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ENDOCRINOLOGY

THE BULLETIN OF THE ASSOCIATION FOR THE STUDY OF

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COMPLETE RECOVERY OF GONADOTROPIC SUBSTANCES FROM THE URINE OF PREGNANT WOMEN*†

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MADISON

There are now available several methods which can be relied upon for the preparation of pregnancy urine extracts which are highly gonadotropic in character. Many of the processes are outgrowths or modifications of the one described by Zondek (1) for obtaining prolan from urine. One or more of the fundamental steps of the Zondek method are therefore common to subsequently developed procedures. Table 1 gives a synopsis of the essential steps of eight recently published methods. It may be seen that two procedures, concentration of the urine, or hormone, and precipitation of the active principle by acetone or alcohol, are employed in all of these methods.

Concentration of the urine to one-eighth or to one-tenth the original volume is an advantageous procedure, from the standpoint of ease of manipulation, when extracts are to be prepared from large quantities of urine. Dickens (2), Wiesner and Marshall (3), Katzman and Doisy (4), and others effect volume reduction by distillation in vacuo at temperatures ranging from 35° C. to 50° C. Wallen-Lawrence and Van Dyke (5) evaporate the urine to a small volume by placing it in shallow pans before a fan. Volume reduction by such means is of necessity a prolonged procedure and many workers have recognized that this may lead to diminished yield of the active substance which is not only heat labile but is also comparatively unstable in solution. Lejwa (6) obtains the active principle in concentrated form by precipitating it from the urine with ammonium sulphate. Similarly the phosphotungstic acid method of Wiesner and Marshall involves concentration of the active substance through precipitation rather than by reduction of urine volume.

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†Read before the Seventeenth Annual Meeting of the Association for the Study of Internal Secretions, Milwaukee, June 13, 1933.

Adsorption of the hormone directly from the urine, as employed in the norit-phenol and in the benzoic acid-acetone methods of Katzman and Doisy, serves both to concentrate the active substance and to remove some of the urinary constituents which are toxic to the injected animals. Permutit adsorption from a water solution of the ammonium sulphate-hormone precipitate is used by Lejwa chiefly as a means of obtaining the hormone in a more highly purified state, since, by dilute ammonia elution, the gonadotropic factor is selectively extracted from the adsorbent. Liberation of the active material from the adsorbent or precipitant is accomplished by means of dilute ammonia in the phosphotungstic acid method of Wiesner and Marshall as well as in the Lejwa process. Katzman and Doisy find that 90 per cent phenol serves to effect its extraction from norit or activated charcoal. In the benzoic acid-acetone method of Katzman and Doisy, the benzoic acid is dissolved in an excess of C.P. acetone and decanted from the gonadotropic acetone insoluble material.

Alcohol or acetone precipitation is used in the majority of extraction methods as a means of securing the hormone in a highly concentrated, very stable form from which water or saline suspensions may be prepared for injection. The precipitation is usually accomplished by the addition of three or four volumes of acetone or alcohol. This brings the final concentration of the precipitant to 75 or 80 per cent. In both the norit and the benzoic acid adsorption methods of Katzman and Doisy a higher concentration of acetone is used. Lejwa is primarily concerned with the preparation of a crystalline material hence employs alcohol or acetone solutions of successively increasing concentration as a means of purification.

In this laboratory six of the eight methods presented in Table 1 have been tested. The extracts were repeatedly found to be less potent than the urine from which they were prepared. The investigators who offer the methods make no claims, however, for total recovery of the active principle. Emphasis is placed on purity of product. Few data are cited as to the percentage recovery of hormone in the extracts as compared with the potency of the whole urine. Results of experiments demonstrating the actual yield are usually reported as units per liter of urine with no reference to activity previous to extraction. If the extraction methods are to be applied to the study of normal cycles, the menopause, and disturbances of menstruation, which is our objective, quantitative recovery of the active principle is of greater significance than is purity of product. The problem thus resolves itself into an investigation of the various methods used, to determine at which point and under what conditions loss or inactivation occurred.

Experiments were conducted which covered possible loss through concentration or temperature inactivation, acetone precipitation, adsorption, and elution from the adsorbent. The experimental findings were utilized to develop a method for preparing extracts of gonadotropic substance from pregnancy urine. The amount of active principle demonstrable in the

TABLE 1

PUBLISHED METHODS FOR PREPARATION OF A-P-L EXTRACTS
(From Pregnancy Urine)

Originators and Method	Summary of Extraction Procedures
Dickens	
*Alcohol Precipitation.....	Volume reduction in vacuo at 40°–50° C. Final precipitation of hormone by ethyl alcohol at 75%.
Wiesner-Marshal	
*Alcohol precipitation.....	Volume reduction in vacuo at 35° C. Final precipitation of hormone by ethyl alcohol at 76%.
*Phosphotungstic acid.....	Hormone precipitation from urine by phosphotungstic acid. Selective solution of hormone from tungstate in dilute ammonia. Final precipitation of hormone by 63% ethyl alcohol plus 1.66% acetone.
Katzman-Doisy	
*Alcohol Precipitation.....	Volume reduction in vacuo at 40° C. Final precipitation of hormone by ethyl alcohol at 75%.
*Norit-phenol.....	Adsorption of hormone by stirring urine with norit. Elution from absorbent by means of 90% phenol. Final precipitation of hormone by ethyl alcohol or acetone at 89%.
Benzoic-acetone.....	Hormone adsorption on benzoic acid in acetone solution. Final precipitation of hormone by redissolving benzoic acid in excess acetone.
Wallen-Lawrence & Van Dyke	Evaporation of urine to small volume in open pans before a fan. Final precipitation of hormone by ethyl alcohol at 75%.
*Lejwa.....	Precipitation of hormone by ammonium sulphate. Permutit adsorption of hormone from water solution of ammonium sulphate precipitate. Elution from permutit with 0.125% ammonia. Final precipitation of hormone by ethyl alcohol or acetone at ?%.

*Active extracts prepared by these methods have been found less potent than the urine from which prepared.

original urine has been recovered in the extracts without appreciable loss and there is no toxicity when the equivalent of 1000 cc. of the urine is injected into 24-day-old female rats.

EXPERIMENTAL

Urines Used. Composite lots of urine from women probably less than two months pregnant were used. After testing the individual specimens by the rabbit ovulation test for pregnancy, those found positive were pooled, preserved with boric acid, and kept at 8° C. until three or more liters were accumulated. A portion of each composite batch of urine was washed four times with ethyl ether to free it from follicular hormone and then injected subcutaneously, in graded doses, into immature female rats for the purpose of determining the minimal effective dose (M.E.D.). Each test dose of urine was diluted with the necessary amount of distilled water to bring the total volume of fluid injected to 0.5 cc. per animal. The M.E.D. of the different lots of urine varied from 0.5 cc. to 1 cc.

Based on the findings reported in the following section, the M.E.D. of urine is defined as the least amount of that urine which, injected subcutaneously in five daily portions, produces in at least one of three, 35 to 45 gram female rats, 24 days of age at the beginning of the injections, a 100 per cent increase in ovary weight (20-25 mgm. ovaries) and one or more corpora lutea at necropsy on the sixth day.

Bioassay. Immature rats, of the Wistar strain and of the Sprague-Dawley strain, were used as test animals. It is well known that, in 21 to 25-day-old female rats the ovarian response to gonad-stimulating materials is extremely variable. This increases the difficulty of attempting to detect quantitative recovery of small amounts of the hormone. Friedman (7) suggests that differences in response are due to the fact that the ovaries of young rats may not all be in the same state of development. It has been noted that, in rats of the two strains used in this laboratory, there is a body weight increase of from 5 to 15 grams between the twentieth and the twenty-fifth days of life. Wallen-Lawrence and Van Dyke (5) point out that there is some correlation between ovary weight and body weight of immature rats. It seems not unlikely that the morphology of the ovaries also might parallel, to a certain extent, the general physical development of the animal as indicated by its weight. Four days is a relatively long period in the life of a young rat. This, too, might account for the lack of uniformity in the ovaries of 21 and of 25-day-old animals. An effort was made to roughly standardize the normal uninjected females of our rat colonies. For this purpose 21-day-old rats, weighing 25 to 30 grams, were separated into five groups of 10 animals each. These animals weighed from 35 to 45 grams on the twenty-fourth day of age. Beginning at the twenty-sixth day of life, one group was killed on each of five successive days. The ovaries, freed from tubes and bursae, were observed for follicular development and weighed in pairs. The animals of the 26-day group exhibited much greater variation as to ovary weight and structure than did those of the group killed at 30 days of age. Another series of 50 rats, weighing 35 to 45 grams at 24 days of age, killed on the thirtieth day of life possessed ovaries which were remarkably uniform as to weight and morphology. In two animals the ovaries weighed slightly more than 16 milligrams; in 44, the ovaries weighed from 10 to 12 milligrams; and in four, the weights were 8 to 9 milligrams. There were a few small follicles in all of the ovaries but no other evidence of morphological development.

These findings were interpreted as indicating that, in 24-day-old rats of 35 to 45 gram weight, the ovaries are in a fairly uniform state of development and may be expected to respond similarly to a given dose of gonad-stimulating substance. To test this a group of 50 rats of that weight range and age were injected with the M.E.D. of pregnancy urines, titrated for potency as previously described. One-half of the animals were given the test dose in five injections, one each day, while the other 25 received the same total amount of urine in 10 injections, two each day for five days. The tests were run in triplicate, using three, four and five animals per injection group. Those in which the ovarian reaction was an approximate duplication of that resulting from the same amount of urine injected in other animals, used for titration of potency of that batch of urine, were recorded as reacting positively. Upon analysis of the data it appeared that, with the injection of the M.E.D. of pregnancy urine, dual injection technic

had no advantage over the single injection procedure. Of the 50 injected animals 39 (78%) responded positively to minimal effective doses of pregnancy urine. In four of the twelve groups the results were 100 per cent positive. There were two negatives in one group of three animals, while in the other groups composed of three rats each there were no negatives. In none of the 12 groups of injected animals did all of the members fail to respond. These data seem to indicate that of three rats, weighing 35 to 45 grams at 24 days of age, upon injection once daily for five days with a test material, at least one may be expected to give a positive response at necropsy on the sixth day, if the material contains one M.E.D. of gonadotropic substance.

Increased ovarian weight, without consideration of the ovarian structure, would seem to be an inadequate basis on which to judge the effective character of extracts. Unless injections of urine and of extract produce ovaries of comparable lutein and follicular development as well as of comparable weight, there would be no assurance that the nature of the hormone constituent had not been altered in extraction. Many investigators have used luteinization as the criterion of potency of their extracts. This may serve well as a qualitative test but is unsatisfactory from the standpoint of quantitative evaluation of gonadotropic content. In this laboratory the titration of composite pregnancy urines has shown that luteinization of a diffuse type usually results from the injection of much smaller amounts than the M.E.D. of urine. Often such ovaries, although almost completely luteinized, weigh no more than do those of normal uninjected control rats of the same age. Progressive increase in the amount of urine injected results in a parallel increase of ovary weight but seldom effects a noticeable change as to type of luteinization until the urine is injected in such an amount as to cause a 100 per cent increase in ovary weight (20-25 mgm. ovaries). At this level of injection, discrete corpora lutea are usually observed in the majority of the injected animals.

Although vaginal canalization and oestrus have never been observed in untreated rats of our colonies as early as the thirtieth day of life, the occurrence of this phenomenon cannot be utilized as a test for the quantitative recovery of gonadotropic material in extracts of pregnancy urine. It is not uncommon to find that with complete and rapid luteinization and extreme ovarian hypertrophy, vaginal opening and oestrus do not occur. On the other hand, establishment of the vaginal orifice with a smear of scales lasting two or three days is frequently associated with the presence of but one or two large follicles in ovaries otherwise immature as to morphology and weight.

In the following experiments the assay of extracts as to potency and character is based on approximate duplication of the effects of the injection of the M.E.D. of whole urine and of the injection of an equivalent amount of the extract prepared from that urine. Inclusion of the factors of animal standardization together with both weight increase and morpho-

logical development of the ovaries imposes rather rigid limitations on the assay procedure. The observation that, under such conditions, much of the variation in response of immature rats to small doses of gonad-stimulating substance is ruled out would seem to justify the acceptance of this assay procedure as a fairly reliable test for the quantitative recovery of the hormone in extracts of pregnancy urine.

Activity Loss Through Concentration. For this experiment a five-liter batch of composite urine which had been found, by rat titration, to contain one M.E.D. of active substance per cubic centimeter was used. One liter aliquots were concentrated under reduced pressure at varying temperatures to one-eighth or one-tenth the original volume. The concentrate from each was separated from the residual solids by suction filtration and poured into 20 volumes of C.P. acetone. The acetone insoluble material from each liter of urine was suspended in water and injected in graded urine-equivalent doses to determine the level at which ovarian response was approximately the same as that resulting from the injection of the M.E.D. of the urine. The results are presented in Table 2. Computation

TABLE 2

LOSS OF ACTIVITY THROUGH CONCENTRATION
(Volume Reduction in Vacuo to 1/10)

Concentration Procedure	Percentage Loss
Distillation at 25°–27° C. for 7 hours	50%
Distillation at 28°–30° C. for 8 hours	50%
Distillation at 40°–44° C. for 3 hours	50%
Distillation at 57°–60° C. for 2 hours	95%

Control: The active principle precipitated from unconcentrated urine by addition of 20 volumes of C. P. acetone was found to retain the original potency of the whole urine.

of the percentage loss of activity in each case is based on a comparison of the M.E.D. of extract and of urine. It may be seen that there is considerable loss of activity through concentration of the urine by low temperature evaporation. This is apparently not a matter of heat inactivation at temperatures up to 44° C., since the percentage loss is approximately the same whether volume reduction be accomplished at 25° to 27° over a period of seven hours or at 40° to 44° for three hours. Greater loss resulted from concentration at 57° to 60° temperature. Decrease of potency following concentration of the urine seems to be largely a matter of retention of the active substance, possibly through adsorption, by the urinary solids from which the concentrate is removed. Repeated washing of these salts with 33 per cent acetone, acidified to pH[5] with glacial acetic acid, yielded significant amounts of the hormone with each of five washings.

Activity Loss Through Acetone Precipitation. The use of 20 volumes of acetone for the precipitation of the active principle from the urine concentrates and from the whole urine, in the experiments described above, is based on experimental findings of which the data of Table 3 is representa-

tive. Precipitation of the active principle by addition of sufficient C.P. acetone to bring the final acetone concentration, of one liter aliquots of pregnancy urine, to 94 or 95 per cent (16 volumes of acetone added at 38° C. or 20 volumes at room temperature) effects precipitation of all of the acetone insoluble material of the urine. No precipitate forms with further addition of acetone to the supernatant solutions. The injection of M.E.D. equivalents of a water suspension of the 94-95 per cent acetone insoluble material produces ovaries which correspond in weight and morphology to those of the experimental animals receiving the M.E.D. of whole urine. The use of this large volume of acetone in the precipitation procedure also serves to separate the estrogenic material from the gonadotropic substance. There is no effect on the uteri of immature castrated female rats injected with 100 cc. urine-equivalents of water suspensions of this material.

Supernatant solutions resulting from precipitation of the active principle at 89 per cent and at 80 per cent acetone concentration were found to contain considerable amounts of material which could be precipitated by the addition of sufficient acetone to bring the final concentration to 95 per cent. That there is slight but appreciable loss of activity, with precipitation at 89 per cent and 80 per cent acetone concentrations, is evident more in the morphology than in the lower weight levels of the ovaries of rats receiving M.E.D. equivalents of water suspensions of these precipitates. Very few discrete corpora lutea are encountered. There is marked luteinization of the diffuse type, which phenomenon is characteristic of injections of subminimal doses of untreated urine. Follicular development is more prominent and the follicles are large and clear with little peripheral luteinization. The 89 and 80 per cent acetone soluble material contained in the supernatant solutions proved to be definitely gonadotropic in character. The precipitates formed upon bringing these solutions to 95 per cent acetone concentration were suspended in water and injected in 100 cc. urine-equivalents into each of three test animals. In all cases ovarian weight was markedly increased and there were blood points as well as excessive luteinization of the discrete type. As would be expected, the activity of the 89 per cent acetone soluble substance was less than that of the 80 per cent acetone soluble.

The addition of C.P. acetone directly to the whole urine forms a precipitate which carries not only the gonadotropic principle but also a large amount of material which is toxic to immature rats injected with 50 to 100 cc. urine-equivalents of the extracts.

Studies of Adsorption Procedures. Complete adsorption of the hormone from pregnancy urine, acidified to pH^5 with glacial acetic acid, was obtained upon shaking one liter of the urine for two hours with 20 grams of either Lloyd's reagent or with norit. After readjustment to pH^5, the adsorption product was separated from the urine by suction filtration and dried on the filter. Filtration through ordinary filter paper has in a few

TABLE 3

Loss of Potency Through Acetone Precipitation

Acetone Precipitation		Assay Data										
		Acetone-insoluble Material						Acetone-soluble Material					
			Ovarian Findings						Ovarian Findings				
Final Concentration	Volumes Added	Amount Injected (Urine-equivalent)	Weight (mgms)	Follicles		Luteinization		Amount Injected (Urine-equivalent)	Weight (mgms.)	Follicles		Luteinization	
				Clear	Lutein Border	Diffuse	Discrete			Clear	Lutein Border	Diffuse	Discrete
95% (Room temperature)	20	0.5 cc.	20.2 19.9 23.8	0 0 0	+ ++ 0	0 + 0	+++ + +++	.....	.....	.....	.....	.....	.....
94% (38° C.)	16	0.5 cc.	22.8 18.7 21.4	0 + 0	+ + +	0 ++ +++	+++ 0 ++++	.....	.....	.....	.....	.....	.....
89%	8	0.5 cc.	18.4 17.8 19.2	0 ++ 0	+ 0 ++	+++ ++++ ++++	0 + 0	*100 cc.	24.7 26.2 23.8	0 0 0	++ ++ ++	+++ +++ ++	++ ++ +
80%	4	0.5 cc.	20.0 16.4 17.1	++ ++ ++	0 0 0	++++ +++ +++	0 0 0	*100 cc.	53.2 48.0 47.6	+ + ++	0 0 0	0 0 0	++++ ++++ ++++

*Many blood points present in all ovaries of these groups.

instances resulted in adsorption of traces of the active principle. As a precaution against such loss, hardened filter paper, Schleicher and Schüll, No. 575, which has been moistened with 1 per cent acetic acid, may be used. The filtrates, after this adsorption procedure, were inert in 5 cc. amounts. Little, if any, ovarian reaction could be detected after the injection of the equivalent of 100 cc. of the filtrate into immature animals. Failure to readjust the adsorption product to pH^5, prior to filtration, resulted in filtrates of significant gonadotropic activity at 100 cc. injection level. Permutit did not so effectively adsorb the active substance. The filtrates, after permutit adsorption, were found to be gonadotropically active even at 5 cc. injection level and the injection of the equivalent of 100 cc. of the filtrate resulted in marked ovarian development. Much of the toxic substance remained in the urine after removal of the hormone by adsorption on Lloyd's reagent, norit, or permutit.

Complete removal of the hormone from the urine could also be accomplished by smaller amounts of Lloyd's reagent. This, however, necessitated a longer period of continuous shaking with the adsorbent. An adsorption period of eight hours was required to effect complete removal of the active substance from one liter of urine by 10 grams of Lloyd's reagent. Injections of 5 cc. of the filtrates obtained after two hours and after four hours of adsorption with this amount of reagent provoked marked ovarian response in the test animals. The six-hour filtrate, although inactive at the 5 cc. injection level, retained sufficient active substance to produce two or three large follicles in the ovaries of rats injected with the equivalent of 100 cc. of the filtrate. The eight-hour filtrate was inert both at 5 cc. and at 100 cc. injection levels. The gonadotropic material was not completely adsorbed by 2.5 grams nor by 5 grams of Lloyd's shaken with one liter of urine for eight hours. No ovarian response was elicited by the injection of 5 cc. portions of these filtrates but the injection of the equivalent of 100 cc. resulted in ovaries of increased weight, with several partially luteinized follicles.

Studies of Elution Procedures. Although Lloyd's reagent and norit appear equally efficient as adsorbents of the gonadotropic factor from urine of pregnancy, they differ greatly as to the ease with which complete elution from the adsorbent is effected. Neutral agents such as ammonia-free distilled water, 33 per cent acetone, and 50 per cent acetone served to extract 50 per cent or more of the active substance from Lloyd's reagent but had little effect on the norit-hormone combination. Dilute alkali, 0.1 *N* NaOH, also was of slight value in the extraction of the active substance from norit although this reagent almost quantitatively freed it from adsorption on Lloyd's. Both alkalinized 50 per cent acetone, one part C.P. acetone and one part 0.2 *N* NaOH, and 50 per cent aqueous pyridine were found to completely extract the adsorbed hormone from Lloyd's reagent while neither of these solutions effected satisfactory recovery from norit. Table 4 incorporates data from experiments designed to compare the ex-

traction value of these two solutions as applied to Lloyd's reagent and to norit adsorption products. It may be seen that, after adsorption of the active principle on Lloyd's, two extractions by shaking continuously with one liter of 50 per cent alkaline acetone for four hours or with the same amount of 50 per cent aqueous pyridine for eight hours per extraction, yield extracts containing the total amount of active substance from one liter of urine. The ovarian response of immature rats receiving M.E.D. equivalents of the 95 per cent acetone insoluble material contained in the extracts corresponded with that of the test animals receiving the M.E.D. of whole urine. Material similarly prepared with norit as the adsorbent is seen to have provoked relatively little ovarian reaction in the injected animals.

Shorter extraction periods of four or six hours each, with aqueous pyridine, did not so completely free the hormone from the Lloyd's reagent as did two eight-hour extractions. Extraction over night, 10 to 12 hours, with aqueous pyridine, was not detrimental. Prolongation of the extraction period to six hours, with alkaline acetone, produced less potent extracts than those prepared by two extractions of four hours each. Extraction from Lloyd's reagent with a solution of two parts of 0.2 *N* NaOH and one part C.P. acetone did not give as satisfactory yields as did the 0.1 *N* alkaline acetone.

Failure to approximate total recovery of the active principle resulted when elution from 20 grams of Lloyd's reagent was attempted by means of two extractions with an amount of 50 per cent alkaline acetone equal to but one-tenth the original volume of urine (Table 4). Similar extraction with this amount of 50 per cent aqueous pyridine, however, effected very nearly quantitative recovery of the hormone with two extractions of eight hours each. The ovaries of two of the three injected rats showed marked increase in weight as well as several corpora lutea, luteinizing follicles, and blood points. Attempts were made to obtain more complete recovery of the active substance with 100 cc. portions of acetone-pyridine solutions rather than aqueous pyridine for elution. Concentrated pyridine was diluted one-half with C.P. acetone, with 50 per cent acetone, and with 33 per cent acetone. None of these acetone-pyridine solutions yielded as satisfactory recovery as did elution with the same amount of 50 per cent aqueous pyridine.

Three extractions with small amounts of 50 per cent aqueous pyridine proved impractical when 20 grams of Lloyd's reagent were used for adsorption. During the third extraction a foamy, viscous mixture formed, from which the liquid extract could be removed neither by centrifugation nor by suction filtration. This technical difficulty was avoided by reducing the amount of Lloyd's reagent used in the adsorption process. It was possible to completely extract the active principle, adsorbed from one liter of urine on 10 grams of Lloyd's reagent, by three eight-hour elutions. It was necessary to use, for each extraction, only that amount of 50 per cent

TABLE 4

Comparison of Elution Procedures Applied to Lloyd's Reagent and to Norit Adsorption Products

Procedure (Applied to 1 liter of urine)		Assay Data (Ovarian Findings after the Injection of Extract Equivalent to the M. E. D. Urine)								
		Alkaline 50% Acetone Extracts (Two 4-hour extractions)					50% Aqueous Pyridine Extracts (Two 8-hour extractions)				
Adsorption (20 grams of agent)	Elution (cc. agent per extraction)	Weight (mgms.)	Follicles		Luteinization		Weight (mgms.)	Follicles		Luteinization	
			Clear	Lutein Border	Diffuse	Discrete		Clear	Lutein Border	Diffuse	Discrete
Norit	1000	12.0 13.0 9.0	+ + Immature	++ 0	0 ++++ Immature	0 0	10.4 15.4 10.1	+ 0 +	+ +++ +	0 0 0	0 + 0
Lloyd's	1000	21.6 21.0 14.6	++ 0 0	+ 0 +++	+++ ? +++	+ ++++ 0	11.6 18.4 20.8	++ 0 0	++++ ++++ +++	++ 0 0	+ ++++ ++++
Norit	100	8.7 11.0 9.1	Immature ++ Immature	0	Immature 0 Immature	0	10.3 10.1 13.4	+ ++ 0	+ + ++	0 0 ?	0 0 0
Lloyd's	100	9.6 10.0 8.0	+ 0 Immature	0 0	++ ++++ Immature	0 0	11.3 18.6 16.8	++ 0 0	0 ++ +	+ +++ +++	0 ++ ++

aqueous pyridine required to make a thin paste with the adsorbent. The total quantity of elution agent used was greatly reduced. Consequently a much smaller volume of acetone was required in the precipitation procedure.

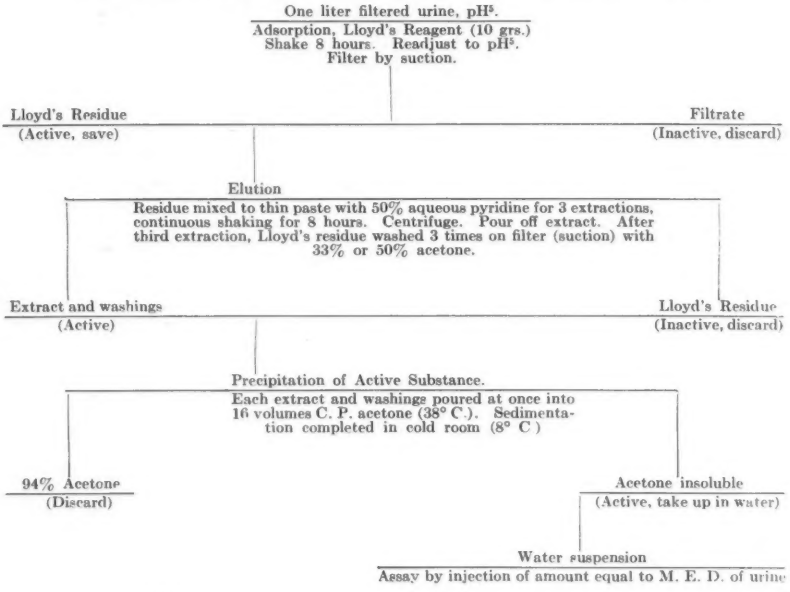
Method for Complete Recovery of Gonadotropic Substance in Extracts of Pregnancy Urine. Based on the results of the early investigations reported here, a method for preparing extracts of gonadotropic substance from pregnancy urine has been offered, in a preliminary publication (8), as a scheme by which two objectives are attained. First, the amount of gonadotropic substance demonstrable in the original urine is recovered in the extract without appreciable loss. Second, non-toxic extracts are obtained so that equivalents of 100 cc. of urine may be injected in 24-day-old rats. If this method were to be applied to the study of normal cycles, the menopause, and disturbances of menstruation as originally intended, further simplification was desired. For these investigations large quantities of urine would be required and this scheme would be cumbersome and expensive due to the large volumes of reagents used for elution and precipitation.

Later studies, directed toward improvement of technic, revealed that with an eight-hour period of adsorption the total hormone content could be removed from one liter of pregnancy urine by but 10 grams of Lloyd's reagent. This modification made possible the complete extraction of the gonadotropic factor by a much smaller total quantity of 50 per cent aqueous pyridine and its precipitation by a greatly reduced volume of acetone. As a result a more practical method has been developed for securing non-toxic extracts without appreciable loss of gonadotropic potency from pregnancy urine. The essential steps of the procedure are indicated in Diagram I. The urine is acidified to pH[5], with glacial acetic acid, and filtered. After shaking continuously for eight hours with 10 grams of Lloyd's reagent per liter of urine, the pH is readjusted to 5 before separating the liquid and the adsorbent by suction filtration. The adsorption residue, dried on the filter, is returned to the extraction flask and intimately mixed with sufficient 50 per cent aqueous pyridine to form a thin paste. The pyridine-Lloyd's mixture is shaken continuously for eight hours. The adsorbent is then packed by centrifugation and the pyridine extract is poured immediately into 16 volumes of C.P. acetone at 38° C. The Lloyd's adsorption product is submitted to three of these eight-hour extractions with 50 per cent aqueous pyridine. Upon removal of the third extract, the Lloyd's residue is washed from the flask, with either 33 or 50 per cent acetone, onto a Büchner funnel prepared with a hardened filter. The adsorbent is dried by suction and washed twice on the filter with 33 or 50 per cent acetone to remove any of the pyridine extract which may have been retained by the residue. The acetone washings are added to the precipitating pyridine extracts. Sufficient C.P. acetone is added to bring the concentration to 95 per cent before placing the precipitation flask in

the cold room (8° C.) to hasten sedimentation. When the precipitate has subsided, the greater part of the supernatant liquid is syphoned off, after which the sediment is packed by centrifugation to permit decantation of the remaining fluid. The acetone insoluble material is suspended in distilled water and placed in the breeze of a fan to evaporate traces of acetone before injecting to test the extract for potency.

DIAGRAM I

METHOD OF PREPARING A-P-L EXTRACTS FROM PREGNANCY URINE


One liter filtered urine, pH⁵.
Adsorption, Lloyd's Reagent (10 grs.)
Shake 8 hours. Readjust to pH⁵.
Filter by suction.
Lloyd's Residue
(Active, save)
Filtrate
(Inactive, discard)
Elution
Residue mixed to thin paste with 50% aqueous pyridine for 3 extractions, continuous shaking for 8 hours. Centrifuge. Pour off extract. After third extraction, Lloyd's residue washed 3 times on filter (suction) with 33% or 50% acetone.
Extract and washings
(Active)
Lloyd's Residue
(Inactive, discard)
Precipitation of Active Substance.
Each extract and washings poured at once into 16 volumes C. P. acetone (38° C.). Sedimentation completed in cold room (8° C.)
94% Acetone
(Discard)
Acetone insoluble
(Active, take up in water)
Water suspension
Assay by injection of amount equal to M. E. D. of urine

Assay of three extracts prepared by this method (Table 5) shows that the original potency of the urine from which they were prepared is recovered in the extracts. No toxic effects resulted from the injection of these extracts in amounts equivalent to 1000 cc. of the whole urine. The injection of the equivalent of 100 cc. of urine was without effect on the uteri of immature castrated female rats. The latter is offered as evidence that the method of extraction effects a complete separation of estrogenic substance.

Not more than 68 cc. of 50 per cent aqueous pyridine per liter of urine, divided in three extractions of eight hours each, was required to secure complete elution of the hormone adsorbed on 10 grams of Lloyd's reagent. In the preparation of these extracts, the total amount of acetone used for precipitation of the active principle did not exceed 1700 cc. per liter of urine. The entire extraction procedure was completed in from 36 to 40

TABLE 5

Complete Recovery of Gonadotropic Activity in Extracts of Pregnancy Urine

Preparation of Extracts (Active principle from 1 liter urine adsorbed by shaking 8 hours with 10 grams Lloyd's Reagent)									Assay of Extracts (Ovarian findings after injection of extract equivalent to M. E. D. of urine)				
50% Pyridine Elution (cc. used in extraction)				Acetone Precipitation (cc. used)					Weight (mgms.)	Follicles		Luteinization	
1st Extract	2nd Extract	3rd Extract	Total	1st Extract	2nd Extract	3rd Extract	Washings	Total		Clear	Lutein Border	Diffuse	Discrete
20	26	20	66	325	425	325	400	1475	15.4 16.7 24.0	0 0 0	++ +++ +++	+ ++++ 0	+ 0 ++
20	24	20	64	325	400	325	500	1550	18.0 24.8 22.7	0 0 0	++++ +++ ++	0 0 0	0 ++ ++++
20	24	24	68	325	400	400	575	1700	21.1 22.0 24.0	0 0 0	++++ ++++ ++++	0 0 0	+ + ++

hours. Judged from the standpoint of ease of manipulation and small quantity of reagents used, this method seems to fulfill the requirements of a practical laboratory procedure for the preparation of non-toxic, estrogen-free extracts without appreciable loss of the gonadotropic substance contained in the original pregnancy urine.

DISCUSSION

From the results of these studies of the fundamental steps of several published methods for the preparation of gonadotropic extracts from pregnancy urine, it would appear that failure to obtain complete recovery of the active principle in the extracts is due to loss rather than to inactivation or destruction of the hormone. In processes employing reduction of urine volume in vacuo, a large part of the active substance is retained by the residual urinary solids from which the urine concentrate used for the extract is removed. Complete precipitation of the hormone by means of acetone necessitates the use of 16 to 20 volumes of the precipitant, a final acetone concentration of 94 to 95 per cent.

Adsorption studies have shown that pregnancy urine, acidified to pH^5 and filtered can be freed of all demonstrable gonadotropic activity by shaking continuously with 1 per cent of Lloyd's reagent for eight hours or more. If 2 per cent of the adsorbent is used, two hours of continuous shaking serves to effect complete adsorption of the hormone. Total elution of the gonadotropic factor is accomplished by repeated extraction with 50 per cent aqueous pyridine or with 0.1 *N* alkaline 50 per cent acetone. The active substance is precipitated upon bringing these alkaline extracts to a 95 per cent acetone concentration. This is contrary to the results of Elden (9) who, in a report published while this paper was being prepared for publication, states that, after adsorption and extraction with 0.1 *N* NaOH, the active material is not precipitated by acetone. Upon adding 16 to 20 volumes of C.P. acetone to the 50 per cent aqueous pyridine extracts, a precipitate forms immediately and has usually completely subsided in from four to eight hours. The final product is an amber colored waxy substance. The precipitation of the active material contained in NaOH extracts, either 0.1 *N* NaOH or 0.1 *N* alkaline acetone, takes place much more slowly. After standing 24 to 36 hours in the cold room (8° C.) transparent monoclinic crystals usually begin to form in the 95 per cent acetone preparations. Complete sedimentation may require 72 hours or more. The final product is an amorphous, dry, white powder. The rate of sedimentation of the active substance from either the pyridine or the alkaline acetone extracts may be hastened by the addition of a few crystals of anhydrous ammonium sulphate or of sodium chloride or by adding a few cubic centimeters of ethyl ether or 95 per cent alcohol to the cold acetone preparations.

Certain findings of the investigations reported here suggest that the presence of acetone delays or inhibits the inactivation of the hormone by

alkali. A higher yield of active substance resulted from elution with 0.1 *N* alkaline acetone than with aqueous 0.1 *N* NaOH. Also, when the 50 per cent aqueous pyridine extracts were evaporated to dryness and the solute taken up in water for injection, according to the procedure used by Fevold, et al. (10) in the preparation of gonadotropic extracts from anterior pituitary tissue, only about 50 per cent of the potency of the original urine was retained in the extracts. Complete recovery of the active principle resulted when the 50 per cent aqueous pyridine extracts were poured immediately into acetone.

Further studies are now in progress with a view to determining whether this proposed method of extraction may be applied to the preparation of gonadotropic extracts from urines of non-pregnant women.

SUMMARY

Various steps in six published methods for the preparation of gonadotropic extracts from pregnancy urine have been studied. Incomplete recoveries are due to losses rather than inactivation. Decreased potency after low temperature evaporation is due chiefly to retention of active material on urinary solids. Acetone precipitations are incomplete unless the final concentration is 94 to 95 per cent.

For adsorption from urine at pH^5 permutit is less effective than norit or Lloyd's reagent. Continuous shaking for eight hours is needed when the Lloyd's reagent used is 1 per cent of the urine weight, but with 2 per cent of the solid the continuous shaking may be reduced to two hours. Total elution of the gonadotropic factor from Lloyd's is accomplished by repeated extractions with 0.1 *N* alkaline 50 per cent acetone or with 50 per cent aqueous pyridine. Neither of these solvents gave complete elution from norit.

A method for preparing extracts of gonadotropic substance from urine of pregnant women is proposed. This involves shaking acidified urine with Lloyd's reagent, elution of the dried residue with 50 per cent aqueous pyridine, precipitation of the active material by acetone at 95 per cent, and suspension of the active solids in water for injection. Extracts prepared by this method have been assayed with controls of whole urine. Approximately duplicate ovarian effects are secured with equivalent doses. This is offered as evidence of satisfactory total recovery of active material. The injection of the equivalent of 1000 cc. of urine gave no toxic effects. The extracts are apparently free from follicular hormone since they have no effect on the uteri of immature castrated female rats.

A modified method of bioassay has been developed which eliminates much individual variation in the response of immature rats to gonadotropic substances. Rats weighing 35 to 45 grams at 24 days of age are injected once daily for five days. At necropsy on the sixth day the ovaries are examined for increases of 100 per cent in weight and for corpora lutea.

Titration of minimal effective doses with small numbers of test animals is facilitated by this scheme.

The writer wishes to express grateful appreciation to Dr. E. L. Sevringhaus, under whose guidance this work has been done, for his helpful suggestions, criticisms, and continued encouragement throughout the work; and also to Dr. F. L. Hisaw and to Dr. H. L. Fevold, whose interest and valuable advice have contributed much to the progress of the investigation.

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THE CLINICAL USE OF PROLACTIN*

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Prolactin, prepared and assayed by Bates and Riddle as previously described (1), in sterile solution at pH 8.0-8.4 was injected intramuscularly in the buttocks. The dose varied from 2.5-10.0 cc. (75-400 units). When the dose was divided the second injection followed within 12-24 hours.

The subjects were 37 women delivered of normal children at the Sloane Hospital for Women (Table I). All were afebrile except one who had a moderately severe sapremia. Twenty-nine cases were chosen in which lactation had failed to develop adequately by the sixth day (fifth in two cases) or later postpartum. These were cases which, in the clinical experience of the staff, would in almost every instance have failed further to improve in milk production. In 21 of these cases there was a gain greater than 100 gm. of breast milk daily when comparison is made between the day preceding prolactin dosage and the date of the patient's discharge 3-9 days later (good result). In four cases the gain was from 50-100 gm. (moderate result). The amount of increase in these 25 cases ranged between 50 and 400 gm. In four other cases (failures) there was either loss or a gain of less than 50 gm.; in two of these failures the dosage was purposely reduced to one-half the recommended dosage; in the other two the cause is less evident but may be due either to insufficient dosage (only 100 units) or to inherent deficiency of mammary tissue. The amount of milk was determined by careful weighing of the babies before and after nursing, and the amounts recorded for each day. Growth curves of the babies usually ran parallel with the increase in the amount of breast milk, and as a rule there was a definite weight increase beginning on the date of treatment.

In a small group of three cases (4, 10, and 35; at 7-15 days) the amount of breast milk was probably at its maximum level, and further stimulation produced no consistent effect. In five presumably normal cases (28-32) prolactin was used at the first, second or third day postpartum. The data are insufficient to show whether such dosage increased the lactation beyond the normal.

In three cases of premature births (not tabulated) the dosage was also effective.

*Preliminary report.

Prolactin injections produced neither local nor general reactions nor postpartum complications.

There were no caked breasts.

TABLE I

CLINICAL DATA ON SUB-NORMAL AND NORMAL LACTATION IN WOMEN

Case Number	Dosage		Grams Milk in Day		Days After Prolactin (Date of Discharge)
	Prolactin Units	On Day Post-partum	Day Before Prolactin	Day of Discharge	
1	100	6	200	395	7
2	100	5	330	495	9
3	100	6	210	360	6
5	100+50+50	6	230	405	8
6	50+ 50	8	295	50	3
7	50+ 50	9	100	260	4
8	50+ 50	10	140	220	3
9	50+ 50	7	225	280	7
11	75+ 35	8	330	170	4
12	75+ 35	8	200	380	5
13	75+ 35	8	170	320	4
14	40+ 35	21	200	310	4
15	100+ 50	8	170	315	2
16	100+ 50	6	170	375	5
17	75	8	200	310	3
18	100+ 50	6	50	90	6
19	160	7	310	450	6
20	250	10	210	360	3
21	250	7	350	470	7
22	100+100	9	45	280	8
23	100+100	8	310	460	4
24	100+100	8	170	570	3
25	100+100	9	180	260	2
26	100+100	7	35	225	7
27	200+200	6	310	480	9
33	200+200	6	40	150	3
34	100+100	6	210	410	8
36	100	7	120	110	5
37	100	5	20	90	6
4	100	8	420	575	5
10	75+ 35	15	520	550	4
35	100+100	7	380	300	5
28	200+200	3	220	350	8
29	200+200	2	70	190	10
30	200	3	190	400	8
31	200	2	90	300	11
32	200	1	70	600	11

On the basis of these results the clinical dose of prolactin is provisionally considered an initial 150 units usually followed within 12-24 hours by 100 units.

We are indebted to Professor Benjamin P. Watson for extending to us the facilities of the Sloane Hospital for Women. Also to Miss Eleanor McLaughlin and to Miss Mary M. King, Head Nurses, in charge of Nurseries, for their careful observation of these cases and their valuable cooperation.

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ENDOCRINE STUDIES: XLII. A NOTE ON ACROMEGALY WITH THE REPORT OF A CASE

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The striking picture of aberrant growth characteristic of acromegaly, since the days of Pierre Marie has commonly been ascribed to an overproduction of the internal secretions of the pituitary gland. Further, it has usually been regarded as a late manifestation of disturbed glandular function, appearing after closure of the epiphyses has inhibited further longitudinal growth of the long bones. Thus a simple grouping has been to recognize gigantism without acromegaly as the result of hyperfunction during the prepuberal and adolescent years, acromegaly without gigantism as the expression of an overactivity, the onset of which falls in the period of maturity and after epiphyseal closure, while the combination of the two has been interpreted as evidence of a long-continued period of hormonal overproduction beginning in childhood and continuing unchecked into the time of an established maturity. Ultimately, the gland tends to undergo functional involution to an ultimate state of hormonal failure, leaving, however, the striking changes in skeletal development as immutable residua of the earlier status. Tumor growth, at least in proportions recognizable by roentgenographic examination, is a far from inevitable background for these conditions, although in those cases in which surgical intervention has been practiced, tumorous growths are usually reported. Biopsy being impracticable in this situation it is to be regretted that postmortem histological examinations are not more frequently obtainable.

Recently the radiographic and anatomical study of a series of skulls from known cases of pituitary disease (1) by one of us (H. M.) has indicated the need for some revision in the simple classification given above. While the majority of the hyperpituitary cases will follow broadly the general grouping as initially stated, the appearance of varying degrees of acromegalic cranial changes in individuals entering the second decade of life and with demonstrably open epiphyses, calls for a recognition of an earlier onset of the characteristic skull changes in at least a portion of those individuals who at some time in youth have a transitory period of pituitary hyperfunction. The term "sub-acromegaly" ("forme fruste") might properly be applied to this single category and the study of such individuals later in life, when the gland has normalized or passed to a typical hypofunctional level, offers, with the latter group, at least, the interesting possibility of a reconstruction of the glandular history of the individual. Our added knowledge of today serves but to add emphasis to the fact, long recognized, that the chronology of the glandular malfunction,

in terms of the growth and developmental possibilities of the individual, plays a primary rôle in determining many of the more salient changes which characterize the varied end results of the putatively common cause.

With this brief preliminary statement, a short report may be made of a partial study recently carried out on a typical case of acromegaly. The patient in question, J. E., is a young man of 26, well-known in professional

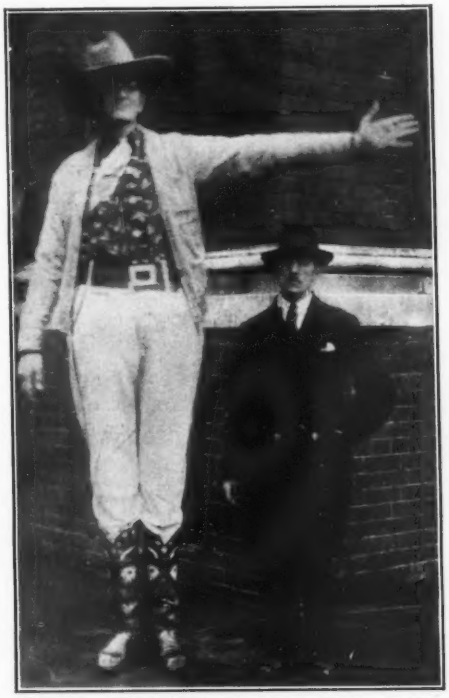

Figure 1. The patient with a man of average size.

circles, where he has appeared as a giant for a number of years. While fulfilling an engagement in Boston, he was kind enough to give us an opportunity for study at such times as his professional activities permitted.*

No formal history was secured from the patient, partly through lack of time and further as it is anticipated that he will be thoroughly studied at a later date. He reported the fact, however, that his principal period of rapid growth began in his eighth and terminated in his sixteenth year. Minor ailments of childhood were reported but none of apparent significant association. He was athletic during his school days and participated in various competitive sports. Football was forbidden on the grounds of possible injury, a most wise interdiction as the radiographic examination of the long bones makes clear.

*It is with great pleasure that we acknowledge our indebtedness for the cooperation, at no little personal inconvenience, which made these records possible.

A thorough physical examination was likewise postponed to a later date. The present study was largely confined to the purely objective data as conforming best to the patient's freedom of time and his convenience. The results may be briefly recorded under the several suitable captions:

Physical Measurements. The more significant of these may be presented in tabular form.

TABLE I
PHYSICAL MEASUREMENTS

Standing Height	228.6	cm.
Span	236.2	cm.
Sitting Height	110.5	cm.
Sitting Height, Index	0.483	cm.
Head, Circumference	60.5	cm.
Neck, Circumference	43.5	cm.
Chest, Circumference (exp.)	119.0	cm.
Chest, Circumference (insp.)	129.5	cm.
Waist, Circumference	107.0	cm.
Hips, Circumference	130.0	cm.
Weight	163.3	kg.

The sitting height index is low, a usual finding in all cases in which the great height is due to undue longitudinal overgrowth of the long bones. By the Dreyer standard of so-called trunk height, the patient is 33 per cent above his predicted weight. Dreyer's subjects, however, were individuals of relatively normal bodily configuration, and the short trunk length influences the prediction unduly. Calculating the appropriate trunk length by multiplying the standing height by 0.525, the normal average index, his predicted and observed weight fall within 3 per cent of each other, a record far more in accord with the facts.

The hands were 25.4 cm. long, the feet 33.0 cm. Numerous other measurements showed relatively normal mutual relationships.

Urine. The output for 24 hours was 3720 cc. with a specific gravity of 1.009. A minimal trace of albumin was recorded and the indican content was notably high. Examination of the sediment showed from 4 to 8 leucocytes and from 2 to 4 hyaline casts per high power field. An occasional blood disc and rare finely granular cast were likewise reported. The urine gave evidence of some degree of renal irritation, the suggestion receiving confirmation from the blood chemistry, as will be shown later. The nitrogen partition examination gave the following results:

TABLE II
URINARY NITROGEN PARTITION

Substance	Gms.	Per Cent
Total Nitrogen	14.37	....
Urea Nitrogen	11.22	78.1
Uric Acid Nitrogen	.29	2.0
Ammonia Nitrogen	.56	3.9
Creatinin Nitrogen	.74	5.1
Residual Nitrogen	1.56	10.9

The protein exchange is certainly not high for a man weighing 163 kilograms but still is probably at a maintenance level. The high residual nitrogen correlates with the evidence of renal impairment. Urea, naturally, is slightly low, the other values relatively normal.

Blood Morphology. The haemoglobin was 88 per cent (Dare) and the red count, 4,980,000, determining a color index of 0.88. The leucocyte count was 9500, while the differential count is given in the next table.

TABLE III
DIFFERENTIAL LEUCOCYTE COUNT

Neutrophiles		43.0
Lymphocytes—		
Small	39.5	
Large	2.0	41.5
Endothelials—		
Trans	7.0	
Mono	0.5	7.5
Eosinophils		7.5
Basophils		0.5

The slight lymphocytosis is a usual finding in the various endocrinopathies and the eosinophilia is characteristic of the various types of pituitary disorders. The coagulation time (Brodie) was the low normal value of 2.5 minutes.

Blood Chemistry. The somewhat thorough study of the blood was made by Dr. Richard Wagner, to whom the authors express their appreciative thanks.

TABLE IV
BLOOD CHEMISTRY

Constituent	Amount
Cells	38.7 %
Plasma	61.3 %
Total Nitrogen (plasma)	1.34%
Albumin	4.58%
Globulin	3.33%
A: G Ratio	1.38
Fibrinogen	0.44%
Non-Protein Nitrogen	40 mgm.
Urea Nitrogen	19 mgm.
Uric Acid	7.5 mgm.
Sugar	117 mgm.
Sodium Chloride (plasma)	646 mgm.
Inorganic Phosphorous	3.6 mgm.
Calcium (serum)	10.2 mgm.

But few comments are necessary. The serum albumin has a low normal value while the globulin is definitely high. This lowers the ratio significantly and offers another evidence of potential renal impairment. This conclusion of lowered renal permeability is further supported by the somewhat high values for the non-protein and urea nitrogen and the plasma chlorides. The high blood uric acid, both absolutely and relatively greatly

increased above the slight increments of the foregoing constituents, is referable to the pituitary condition and characteristic of it among the endocrinopathies. The blood sugar is a high normal, an observation referable either to the nervous instability of the patient or evidencing a tendency toward a hyperglycaemia that may be remarked in pituitary hyperfunction. In the present instance we incline to the first explanation as objective evidences are lacking to define a present hyperactive condition of the gland.

Figure 2. Lateral sklagram of skull.

On the contrary, it is apparently in a state of downward functional transition. The other constituents fall within the conventional limits of the normal.

Respiratory Metabolism. Some little difficulty was experienced at first in securing this datum as the large volume of the respiratory exchange precluded the use of any of the usual closed circuit types of apparatus

which constitute our usual practice. By adopting the open circuit approach and using Tissot spirometers of 125 liter capacity, a series of reasonably satisfactory measurements were secured. During the preliminaries the patient told us that a basal rate measurement had been attempted upon him some years previously. It was abortive as obviously a closed circuit apparatus was used and he exhausted the spirometer in a brief space of time with results which had apparently disturbed him. This recollection, together with his own nervous instability, undoubtedly influenced adversely the results of our own measurements. His first observed rate was 3245 calories with an R.Q. of 0.97; the second attempt yielded a value of 3107 with the more plausible coefficient of 0.89, and the third and last measurement gave a value of 2969 cal. based upon the R.Q. of 0.89 again observed or 2916 cal. if the conventional practice of assuming an R.Q. of 0.82 be followed. In our opinion, these results give undoubted evidence of the influence of an emotional factor which precluded the measurement of the true basal level in this case as it has in so many others encountered during the past decade. Assuming the lowest value 2969 as the most nearly representative value, comparison with the usual standards of prediction give the following results:

TABLE V

Prediction	Amount	Deviation
Harris-Benedict	3279	−9%
Aub-du Bois	3048	−3%
Same, Boothby modification	3110	−5%
Dreyer	3051	−3%

Use of the lower value of 2916 cal. would depress these deviations no more than an inconsiderable 2 per cent. In other words, all of these values fall within the conventional normal limits of deviation from prediction. As noted above, however, the observed value is a maximum and the true basal rate certainly lower although to what degree it is impossible to say and idle to speculate. Further support for this contention is found in the fact that the pulse rate during the measurements was from 78 to 80 per minute, while at other times consistent records of from 58 to 60 were secured. The upward trend in the rate is no more than one other evidence of nervousness during the test, which condition the patient also subsequently affirmed. The respiration rate was 10, the temperature 98.6° and the blood pressure 146 mm. systolic and 90 mm. diastolic.

Carbohydrate Metabolism. It was impossible in the limited time available to secure a complete set of galactose tolerance tests. Only one morning could be given to this and a 20-gram dose was selected as a positive response would show a depressed, a negative test normal or increased tolerance. The results follow:

TABLE VI

GALACTOSE TEST. 20 GMS.

Time of Urine Collection	Sugar	Nitrogen Elimination
5 A.M. to 7 A.M.	0	2.95 gms.
7 A.M. Test Meal administered.		
7 A.M. to 9 A.M.	0	2.71 gms.
9 A.M. to 11 A.M.	0	1.40 gms.

The usual precautions were observed, the test was negative, and the sugar exhibited a protein sparing action so far as the urine nitrogen is to be regarded as defining.

The galactose tolerance was not depressed; the single test fails to indicate if it was normal or increased.

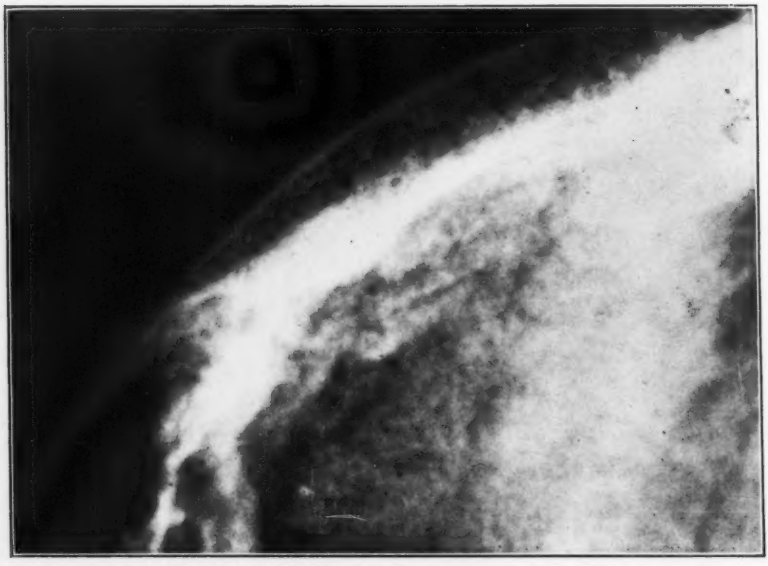

Figure 3. Skiagram of frontal bone above the sinuses.

Eye Examination. The routine examination was carried out by Dr. H. M. Emmons, to whom we express our indebtedness. Vision with glasses was recorded as: right, 10/30; left, 10/10. The pupils were 4 mm. in diameter and were equal and round. Light and consensual reactions were reported as slightly sluggish; accommodation, normal. Of the right fundus it was reported: "The arteries appear normal in size and shape; veins slightly dilated; optic nerve appears slightly pale; disc margin hazy." On the left, "the veins are more dilated than on the right, the nerve head shows a slight papillitis; the optic nerve is slightly pale."

Both fundi show yellowish tinge, the left more pronounced than the right."

The form fields showed a marked upper cutting, recoverable on raising the lids. The color fields and the blind spots were normal.

Roentgenological Examination. The lateral skiagram of the skull shows characteristic "Type 1" acromegalic changes (1); in all the com-

Figure 4. Antero-posterior skiagram of skull.

ponent bones of the head there is evidence of overdevelopment of cancellous bone, as well as overgrowth of those structures normally developed in cancellous bone.

The most striking changes are seen in the frontal bone. The frontal sinuses are huge (Figs. 2 and 3); in fact, they are the largest we have seen either clinically, in roentgenograms, or in the museum material collected

in America and England. Laterally, the sinuses extend to the zygomatic processes of the bone (Fig. 4); vertically, they are extensive, the left sinus measuring 68 mm. in height and reaching about half way to the coronal suture; antero-posteriorly, they are 65 mm. in depth and extend more than half way back in the orbital plate of the frontal bone.

The sphenoid, ethmoid, and maxillary air sinuses (Fig. 2) are very large and the mastoid cells (Fig. 6) are the largest and most extensive we

Figure 5. "Close-up-view" of antero-posterior skiagram of frontal bone, showing sinuses and their extensions.

have seen; there is a continuous mass of pneumatization between them and the frontal sinuses (Fig. 2).

In the calvarium the most marked change is seen in the frontal bone above the sinuses (Fig. 3), and here is revealed the fundamental nature of the bony changes which are responsible for the various deformities, found in this condition. Here the bone measures 13 mm. in thickness, and of this some 9 mm., or five-sixths, is seen to be diploë. The outer table of the bone is clearly defined, but not thickened. The coronal suture is already

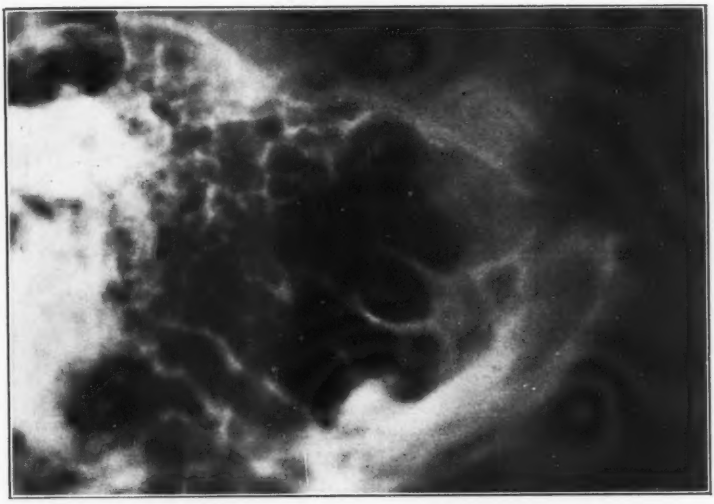

Figure 6. The mastoid air cells.

ossified—the patient is 26. The inner table is not markedly thickened, but there is evidence at certain points of some sclerosis extending into the adjacent diploë.

Figure 7. The symphysis menti.

"The sella turcica (Fig. 2) measures 11 mm. x 14 mm., and the floor is somewhat depressed and irregular, suggestive of intrasellar tumour."

Although prognathism, in the true sense, is not markedly present, there is considerable progenium, and a close-up-view of the symphysis menti (Fig. 7) shows clearly how this result has been produced. The symphysis is seen to be composed of rows of horizontally-placed, thin-walled, expanded cancellous bone spaces—progenium resulting mechanically from the expansion of the constituent elements of the bone, and not in any real sense from deposition or growth of new bone. Indeed, the mandible is obviously

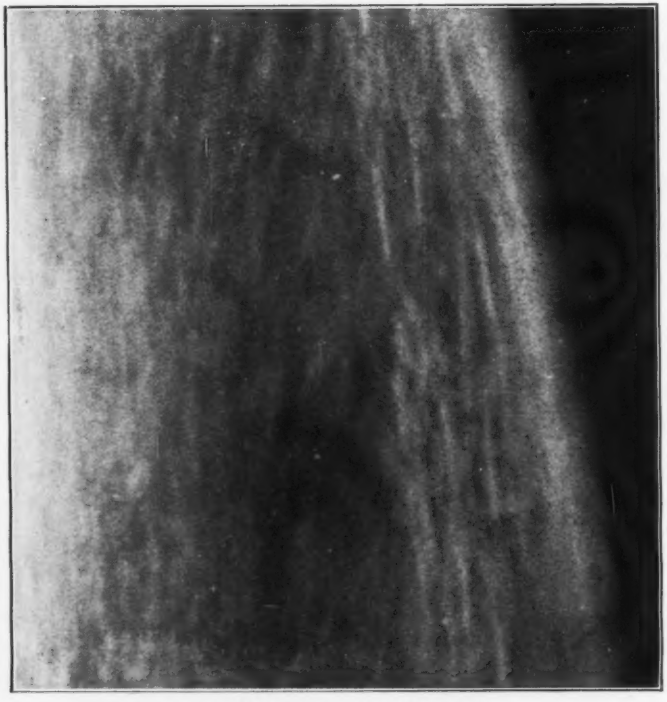

Figure 8. Skiagram of middle third right femur.

rarefied. Although, as already noted, there is hyperpneumatization of the maxillary antra, which has a definite influence on the size of the superior maxilla; the degree to which an antrum may expand is limited by the fact that it is but a single air-cell, whereas, if the cancellous spaces in the mandibular rami expand also to the maximal degree, the total increment in length, so gained, may easily surpass any gain, in a forward direction, of the superior maxilla; thus progenium will result.

The antero-posterior cranial skiagram (Fig. 4) shows clearly the extreme degree of pneumatization of the face. On both sides, above the

upper limits of the frontal sinuses, can be seen areas where overexpansion of trabeculae has caused their breakdown, and the formation of additional sinus-area, the limits of which (Fig. 5) are indicated by the strengthening calcification of remaining, supporting trabeculae. Such areas show the mechanism whereby the anatomical sinuses have reached their present high "growth" in the bone—especially on the left side. This photograph, also, shows the extent to which the frontal sinuses extend laterally into the orbital plates, a fact which, to us, is indicative of early vigorous growth of the sinus.

Figure 9. Skiagram metacarpo-phalangeal joint of thumb.

Skiagrams of the phalanges (Fig. 9) show great longitudinal expansion of cancellous bone. There is no "anchor tufting," nor is there anywhere, in the osseous system of this patient, evidence of either osteo-arthritic or cystic change.

The femoral skiagram is of much interest because of the great length of the bone. From the tip of the great trochanter to the lower end of the internal condyle, the bone measures 80.6 cms. Three 14 x 17 in. films were required to skiagraph this bone with its joints. Figure 8 is a "close-up-view" of the roentgenogram of its middle third, with a celluloid millimeter

scale laid on the film. About three-quarters of the diameter of the bone is shown, as can be seen from the position of the medullary cavity. Noteworthy is the relatively poor proportion of cortical to medullary bone, and the great length of the thin-walled cancellous spaces. One of these, about the middle of the picture, can be seen to measure 22 mm. in length.

From the foregoing it will be seen that in this patient there is one basic change in all the skiagrams—overexpansion of cancellous bone. This accounts for the thickening of the skull, the enlargement of all the paranasal and other sinuses, the jaw deformity, the huge hands and feet, and finally it is this mechanism by which the patient has attained his great height.

SUMMARY

Recent radiographic and anatomical studies in established pituitary cases have demonstrated that acromegalic changes, primarily in the skull, are not confined to adult years. Further, aborted or subacromegalic types have been demonstrated by radiography in children presenting characteristic evidences of pituitary dysfunction. Finally, tumor growth, at least of a magnitude demonstrable by x-ray, is by no means an essential feature of the condition.

In illustration, the paper presents the results of a partial study of a well-known acromegalic giant. Fairly complete physical measurements are supplemented by blood and urine studies, the respiratory and carbohydrate metabolisms and an eye examination. Certain features, as the low sitting height index (0.483), bulky urine (3720 cc.), eosinophilia (7.5 per cent), high blood uric acid (7.5 mgm.), are characteristic diagnostic signs. Typical evidences of renal irritation were presented by the urine (casts, blood discs), the blood chemistry (non-protein and urea nitrogen and chlorides), and a low albumin: globulin ratio (1.38) in the blood. His oxygen consumption was recorded as 9 per cent below the Harris-Benedict prediction and was undoubtedly lower as he was not in a completely basal state. The report ends with a number of the plates obtained during the fairly complete radiographic study. The skull findings were typical of the authors' Type 1, showing hyperostosis of the vault with marked expansion of the diploë and extreme hyperpneumatization of the sinuses; there was well defined asymmetrical prognathism.

The sella was 11 x 14 mm., the floor somewhat depressed and irregular suggestive of possible intrasellar tumor. Other plates give additional skull details and show the hands, femur, etc. Overexpansion of cancellous bone is demonstrated in all areas showing marked overgrowth and is regarded as being the underlying mechanism.

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STUDIES ON THE PHYSIOLOGY OF LACTATION. III. THE RECIPROCAL HYPOPHYSEAL-OVARIAN RELATIONSHIP AS A FACTOR IN THE CONTROL OF LACTATION†§

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Investigations during the past few years by Stricker and Grüter (1929), Corner (1930), Nelson and Pfiffner (1930-31), and Turner and Gardner (1931) have established the relation of the anterior hypophysis to the induction of lactation. These various studies have shown that the mammary glands must be developed under the influence of the ovarian hormones before the anterior lobe factor can initiate secretory activity. The degree of development of the glands which is necessary before lactation may be induced and the relative importance of the two ovarian hormones, oestrin (theelin) and progestin (corporin), in the production of gland growth varies in the different species of animals examined. However, it seems definitely established that the mammary glands of any animal must have attained at least the condition present in the adult female of that species before lactation may be induced by the administration of anterior lobe extracts.

These experiments, however, have given us very little information concerning the manner in which the lactation-inducing principle of the hypophysis is related to the initiation of milk secretion in the parturient female. Since it is well known that an interruption of pregnancy, at least during the latter half, is usually followed by lactation it is apparent that the mammary glands have the capacity to secrete milk during pregnancy. Why, then, does lactation not occur during gestation? Is it due to a lack of the principle responsible for lactation or are there other contributing factors such as the placenta or foetus which may inhibit milk secretion?

Halban (1905) states that in parturient women when the placenta is retained following delivery, lactation does not occur during the period of retention. Frankl (1923) implanted placental tissue into rats near term and observed that no secretion occurred following delivery as long as the grafts remained active. These and other similar observations indicate that the placenta may play some rôle in the control of lactation. Aschheim and Zondek (1927) have shown that oestrin is present in the blood of women in quantities increasing with the progress of pregnancy. As it is generally believed at the present time that the placenta is the source of

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the major portion of this hormone during gestation it would seem that oestrin may be the factor which inhibits the initiation of lactation. Inasmuch as it has been adequately demonstrated that oestrin is intimately concerned with the development of the mammary glands we are confronted with the possibility that it may promote growth but inhibit secretion.

De Jongh and Dingemanse (1931) state that they were able to produce mammary growth in male guinea-pigs by the daily administration of 200 R. U. oestrin. When this was rapidly reduced to 2 R. U. lactation occurred. Fellner (1931) has presented similar experiments and interprets them as indicating that oestrin alone promotes the mammary growth of pregnancy, but inhibits lactation by direct action upon the alveoli. He apparently has taken no account of the evidence relating the hormone of the corpus luteum to mammary development and, furthermore, completely ignores the wealth of evidence which has demonstrated the positive character of the direct relation of the anterior hypophysis to lactation.

It is well known that in most animals ovariectomy or excision of the corpora lutea terminates pregnancy. However, it is not invariably the rule in women as has been shown by a number of reports (Waldstein, 1929, and Pratt, 1927). Waldstein was able to show that in a woman ovariectomized on the 34th day of pregnancy the course of gestation was normal and that theelin could be demonstrated in the blood, urine, and placenta. The guinea-pig is a second exception as has been reported by Loeb (1917) and confirmed by Herrick (1928). These workers state that bilateral ovariectomy may be followed by a normal progression of gestation, mammary gland development, labor, and lactation. Ovariectomy is not followed by lactation in pregnant animals if abortion does not occur. Therefore, it would seem that in pregnancy a factor, other than the ovaries, is present which in some way inhibits milk secretion.

Experiments on non-pregnant female and male guinea-pigs have been reported by Nelson (1932) which have been interpreted as indicating the existence of a reciprocal relationship between the anterior hypophysis and the ovaries in the control of mammary gland development and function. This concept is closely related to the idea of reciprocal pituitary-gonad relationship advanced by Moore and Price (1932) for the control of gonad functions. The latter workers have been able to show by a variety of experiments that the gonadal hormones, although requiring the anterior lobe hormones for their secretion by the gonads, have a suppressing action upon the anterior hypophysis. This reciprocal relationship between hypophysis and gonads is believed to be an important mechanism in the control of the sexual cycle.

In the experiments here reported pregnant guinea-pigs have been employed in the majority of instances. It seemed necessary to extend the earlier experiments with non-pregnant animals to the pregnant female in order to test further the validity of the reciprocal relationship hypothesis

when applied to animals with the mammary development designed for normal secretory activity.

In some of the females employed the stage of pregnancy was definitely known through timed observations. In other instances the exact stage was unknown and could only be estimated. Foetal size, nipple length, and data regarding the individual oestrous cycles have all been used in estimating the duration of pregnancy. Since the guinea-pig gestation period, in our experience, ranges from 64-66 days it was possible to figure back in animals carrying their young to term and thus date the pregnancy.

1. INJECTION OF A LACTATION-INDUCING EXTRACT IN PREGNANT ANIMALS

Pregnant animals exhibit a regular progression in mammary gland growth, but do not lactate. The absence of lactation seems to be due either to a lack of the pituitary principle responsible for lactation or to an inhibiting influence acting directly upon the mammary glands. Evidence

TABLE 1

INJECTION OF A LACTATION-INDUCING EXTRACT IN PREGNANT GUINEA-PIGS

Animal No.	Stage of Pregnancy	Injected Daily	Result	Later History
481	34 days	1. cc. x 2	Abortion in 40 hours.....	Lactation 3 days, died
482	39 days	1. cc. x 2	Abortion in 38 hours.....	Lactation 2 days, died
483	40–45 days	0.5 cc. x 2	Abortion in 52 hours.....	Lactation 3 day., died
484	45–50 days	1. cc. x 2	Abortion in 60 hours.....	Lactation 18 days, lived
485	56 days	0.5 cc. x 2	Abortion in 32 hours.....	Lactation 3 days, died
486	30–40 days	1. cc. x 2	Abortion in 40 hours.....	Lactation 14 days, lived
487	40–45 days	1. cc. x 2	Abortion in 24 hours.....	Lactation 2 days, died
488	60 days	1.5 cc. x 2	Abortion in 36 hours.....	Lactation 2 days, died
489	54 days	0.5 cc. x 2	Abortion in 32 hours.....	Lactation 21 days, died

in this regard might conceivably be furnished by administering a lactation-promoting extract of the anterior pituitary to pregnant females. The occurrence of lactation following such treatment would indicate that the absence of lactation during pregnancy is due to relative or complete lack of the lactation-inducing hormone.

Nine pregnant females were injected with an alkaline extract* of sheep hypophysis known to be potent for the lactation-inducing factor. In the following table it will be seen that abortion occurred in each instance and was followed by lactation. In most cases the female died within a few days, but in the remaining instances lactation persisted at least two weeks. In no instance was lactation observed before the occurrence of abortion so these experiments did not furnish evidence concerning the possible induction of lactation in pregnant animals by introducing the pituitary hormone.

*The sheep pituitary extract and theelin employed in this study was generously supplied by Dr. Oliver Kamm of Parke, Davis & Company. The writer sincerely appreciates Dr. Kamm's earnest cooperation.

2. ABLATION OF THE OVARIES IN PREGNANT FEMALES

Twenty-five females were bilaterally ovariectomized during pregnancy, usually in one operation. In some instances one ovary had been removed before the inception of pregnancy, in other cases the ovaries were removed in two stages. As the subsequent history of these several groups showed no significant difference that might be attributed to the type of operation they will be considered together. In the following table it will be observed that 9 of the 25 animals maintained a successful pregnancy to term; gave birth to living, viable young; lactated normally and, with one exception (Animal 324), reared their young. At the time of weaning these females were sacrificed and a careful examination was made for residual ovarian tissue. In no instance was such tissue found.

TABLE 2

ABLATION OF THE OVARIES IN PREGNANT GUINEA-PIGS

Animal No.	Stage of Pregnancy	Type of Operation	Result of Operation	Days From Operation to Delivery
99	60 days	1-stage	1 living young	5
193	52 days	1-stage	Abortion, 3 young	2
194	50–60 days	2-stage	Abortion, 2 young	3
195	40–50 days	2-stage	Abortion, 2 young	8
196	48 days	2-stage	1 living young	17
197	40 days	2-stage	Abortion, 3 young	11
198	25–30 days	1-stage	Abortion, 1 young	2
240	50–55 days	2-stage	1 living young	12
249	40–50 days	1-stage	Abortion, 4 young	13
250	52 days	1-stage	Abortion, 3 young	1
251	43 days	1-stage	1 living young	22
252	30–35 days	2-stage	Abortion, 3 young	13
253	40–50 days	2-stage	Abortion, 4 young	2
254	20–25 days	1-stage	Abort on, 3 young	3a
255	20–25 days	2-stage	Abortion, 2 young	5
256	46 days	1-stage	2 living young	20
257	52 days	2-stage	2 living young	14
339	52 days	2-stage	1 living young	13
322	55–60 day	1 stage	Abortion, 1 young	2
323	55 days	1-stage	Abortion, 2 young	7
324	55 days	1-stage	3 living young (died)	8
331	35–40 days	1-stage	Abortion, 3 young	3a
333	50 days	2-stage	Abortion, 3 young	1
334	35–40 days	2-stage	Abortion, 2 young	15
335	40 days	1-stage	2 living young	25

a—Secreted only colostrum.

SUMMARY

Eight females to term, all operated upon between 40 and 60 days, 1 or 2 young.

One female, 3 young at 63 days (died).

Ten abortions, all operated upon between 40 and 60 days; 6 with 3 or 4 young, 4 with 1 or 2 young.

Six abortions, all operated upon between 20 and 40 days; 3 with 3 or 4 young, 3 with 1 or 2 young.

In each instance the pelvic girdle was relaxed at the time of delivery, whether abortive or at term. In no case did lactation occur following ovariectomy until the uterus was emptied by delivery or abortion. Milk secretion set in at that time in all except two cases. The degree of lactation varied somewhat in the various animals but could be correlated with the stage of pregnancy.

This experiment demonstrates that some factor, other than the ovaries, is present in pregnant guinea-pigs which inhibits lactation. This inhibit-

ing agency seems to be oestrin or at least oestrin in part since it was possible to demonstrate the normal progression of mammary development following ovariectomy. Figure 1 represents curves drawn from the nipple

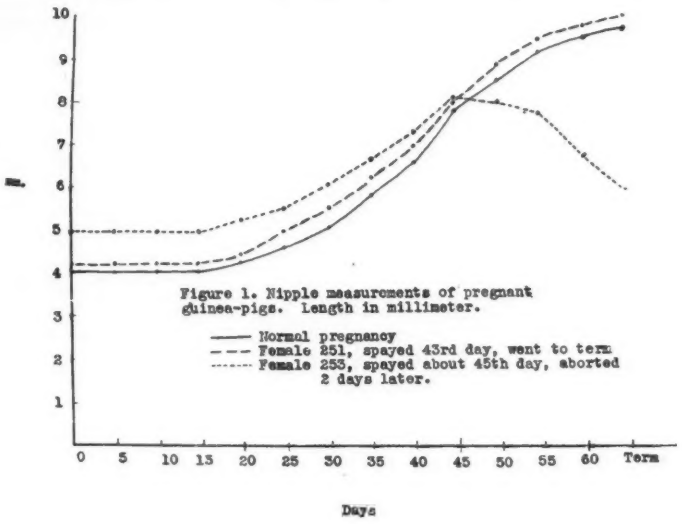


Figure 1. Nipple measurements of pregnant guinea-pigs. Length in millimeter.

measurements of several pregnant females. When pregnancy was maintained following ovariectomy the nipple growth corresponded to that of a normal pregnant female. In the event of abortion nipple growth ceased. We have found that, in the guinea-pig, nipple-growth may be employed, to some extent at least, as a reliable index of mammary gland development.

Attention should be called to two points concerning the cases of successful pregnancy. It will be observed that in these animals ovariectomy was performed after 40 or more days of gestation, and that in no instance did more than two young result from the pregnancy. In the 17 cases of unsuccessful maintenance of pregnancy one animal gave birth to living young which died within a few hours. If the remaining 16 cases are divided into two groups, one comprising the animals operated upon at 40 days or more, and the second embracing those operated upon earlier than the 40th day of pregnancy, it will be seen that there are 10 animals in the first group and six in the second. If the 10 animals operated at 40 days or more are further divided into groups comprising those carrying one or two foetuses and those with three or four foetuses we find four and six animals, respectively. These data demonstrate that pregnancy may continue after ablation of the ovaries in the guinea-pig if the operation is performed after the 40th day of gestation. Of 19 animals operated after 40 days nine gave birth to living young, indicating that approximately 50 per cent of such animals may go to term. If only the females carrying one or two foetuses are included there were eight successful pregnancies and four abortions. The failure of females carrying more than two foetuses to maintain pregnancy to term and the occurrence of eight successful gestations in females with one or two foetuses suggests the possible presence of some threshold relationship which permits the maintenance of pregnancy after the 40th day if only one or two foetuses are present, but does not allow for the maintenance of more than two. It seems apparent that the hormone of the corpus luteum is not essential for the maintenance of pregnancy in the guinea-pig after the 40th day of gestation. It is possible, of course, that the hormone has some other source than the ovaries during later pregnancy.

3. RESULTS FOLLOWING SURGICAL REMOVAL OF THE ENTIRE PREGNANT UTERUS

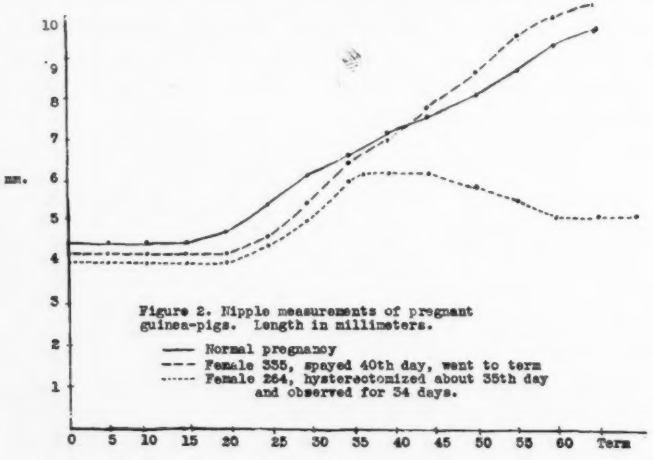
In 12 pregnant animals the entire uterus was removed, leaving the ovaries intact and interposing the least possible interference with the ovarian blood supply. The results of this series of experiments are shown in Table III.

TABLE 3

SURGICAL REMOVAL OF THE ENTIRE PREGNANT UTERUS

Animal No.	Stage of Pregnancy	Number of Foetuses	No Lactation Observed for	Later Treatment and History
212	60 days	3	7 days	Pituitary extract, lactation
258	50–60 days	2	40 days	Sacrificed
259	48 days	2	10 days	Pituitary extract, lactation
260	40–50 days	1	5 days	Pituitary extract, lactation
261	30–40 days	3	12 days	Pituitary extract, lactation
262	42 days	2	7 days	Pituitary extract, lactation
263	25–30 days	2	8 days	Pituitary extract, lactation
264	30–40 days	3	34 days	Sacrificed
265	55–60 days	1	28 days	Sacrificed
266	54 days	2	10 days	Pituitary extract, lactated
325	45 days	1	6 days	Pituitary extract, lactated
329	45 days	2	25 days	Pituitary extract, lactated

No lactation occurred in these animals during periods ranging from 5 to 40 days after the removal of the uterus. Nine of the animals were injected with the anterior pituitary extract and in every instance were induced to lactate. In view of the absence of lactation following hysterectomy in pregnant guinea-pigs it would appear that their ovaries, too, pro-



duce a substance which inhibits lactation. This substance probably is not oestrin since mammary development ceases soon after hysterectomy. Figure 2 represents curves drawn from the nipple measurements of an animal

with normal pregnancy, a hysterectomized and an ovariectomized animal in which pregnancy continued to term. In contrast to the continuation of nipple growth in the ovariectomized female is the cessation of growth in the hysterectomized animal.

4. SURGICAL REMOVAL OF THE UTERUS AND OVARIES IN PREGNANT GUINEA-PIGS

Experiments 2 and 3 demonstrated that lactation does not follow hysterectomy in pregnant guinea-pigs and also does not follow ovariectomy if pregnancy continues. These results have been interpreted as indicating the existence of lactation-inhibiting activities by both the ovaries and some phase of the uterus-placenta-foetus complex.

In five pregnant females the entire uterus and both ovaries were removed. In each instance lactation occurred within 40 hours. The individual results of this series are summarized in Table 4.

TABLE 4

SURGICAL REMOVAL OF THE OVARIES AND PREGNANT UTERUS

Animal No.	Stage of Pregnancy	Number of Foetuses	Result of Operation
267	40–45 days	2	Lactation in 24 to 30 hours
268	50–60 days	3	Lactation in 28 to 36 hours
269	50–60 days	2	Lactation in 28 to 34 hour
270	40–45 days	1	Lactation in 32 to 40 hours
271	35–40 days	2	Lactation in 30 to 36 hours

Thus removal of both ovaries and uterus from pregnant animals permits lactation by removal of the two sources of suppressing influences. Following the operation and initiation of lactation no further nipple or mammary gland growth occurred.

5. SURGICAL REMOVAL OF THE OVARIES AND THE FERTILE UTERINE HORN

The above experiments have demonstrated that some component in the uterus-placenta-embryo complex produces a substance which promotes mammary growth and inhibits lactation. It became of interest to investigate further this complex.

Four females which had been unilaterally ovariectomized preceding the incidence of pregnancy were operated upon between the 40th and 55th days of gestation. The presence of but one ovary in these animals limited pregnancy to one horn of the uterus. At operation the remaining ovary and the fertile horn only were removed. This procedure permitted the sterile horn to remain *in situ*. In each of these animals milk was secreted within 36 hours following operation. It would appear that the inhibitory influence exercised over lactation by the uterus-placenta-foetus complex is not produced in the uterus, at least not in the uterus unaltered by the presence of a foetus.

6. SURGICAL REMOVAL OF THE OVARIES AND EMBRYOS

Six unilaterally spayed females having a pregnancy in one horn of the uterus were subjected to an operation in which the remaining ovary was removed. At the same time the fertile uterine horn was opened and the embryos shelled out. Care was taken not to disturb the placentae any more than was absolutely necessary for the removal of the foetuses. One female died on the second day following operation, but the remaining five recovered. A careful watch was maintained for the delivery of the placentae and in three cases they were found. The remaining two females dropped and devoured them during the night. Table 5 contains the details of this experiment.

TABLE 5

SURGICAL REMOVAL OF THE OVARIES AND FOETUSES

Animal No	Stage of Pregnancy	Number of Foetuses	Period of Placental Retention	Lactation Following Placental Expulsion
275	45 days	2	2 days	30 to 40 hours
276	35–40 days	1	4 days	24 to 30 hours
277a	40–50 days	1	.....	.....
278	50–55 days	2	5 days	30 to 36 hours
279	40 days	2	4 days	38 to 45 hour
280	40–45 days	3	3 days	35 to 40 hours

a—Died.

In no instance did lactation occur during the period of placental retention. The time of its occurrence after expulsion of the placenta is quite comparable to that following abortion in ovariectomized pregnant females. It is true that the placentae were not retained long after the foetuses were delivered, but the period of retention together with the attendant failure of lactation to occur appears to be significant and to indicate that the placenta is the source of the substance produced by the uterus-placenta-foetus complex which inhibits lactation during pregnancy. The evidence indicating this agent to be oestrin has been presented. Furthermore, that the placenta produces oestrin during pregnancy, at least in some species, is a generally accepted assumption at the present time.

Most investigators who have had occasion to examine large numbers of parturient animals have doubtless observed an occasional animal which fails to lactate. Smith and Smith (1933) have recently reported such cases in the rabbit. During the past two years three such cases have come to my attention. In each of these cases the pregnancies appeared to be near term, but all were abortive in character. The foetuses in each instance were dead when found. Two of these females were autopsied after three days and in each instance bits of placental tissue, apparently healthy and well attached were observed. In the third case the female was injected with 6 cc. of the pituitary extract over a two-day period. Following this treatment lactation occurred and the animal was sacrificed. Three placental sites with residual tissue were found.

7. INHIBITION OF LACTATION IN PARTURIENT FEMALES BY OESTRIN ADMINISTRATION

Five normal parturient females were injected with 100-125 R. U. of theelin daily. Injections were begun within a few hours of delivery and continued for 5 to 6 days. In two instances, a scanty lactation occurred the day following parturition, but did not persist. In each case milk secretion was held in abeyance during the injection period but appeared within two days following cessation of treatment. This experiment is further evidence that oestrin inhibits lactation.

Three parturient females were given 125 R. U. of theelin daily for 6 days, but in addition were injected with 3 cc. of the pituitary extract each day. In these animals lactation occurred normally and the young were successfully suckled. This experiment supplements the preceding one and suggests that oestrin inhibits lactation by exerting a suppressing influence upon the production of the lactation-inducing hormone by the anterior hypophysis. However, when 300 R. U. of theelin was administered daily together with 5 cc. of pituitary extract (2 animals) only a scanty lactation occurred over a five-day period. When the theelin was dropped to 100 R. U. lactation increased. This experiment indicates that oestrin, when present in large amounts, may inhibit lactation by acting directly upon the mammary glands as well as by suppressing the output of pituitary hormone.

8. THE EFFECTS OF CASTRATION AND SUCKLING ON THE MAMMARY GLAND

It seems apparent from the foregoing experiments that oestrin is an important factor in the suppression of lactation and that an anterior-pituitary hormone is active in its initiation. There remains to be considered the maintenance of secretory activity. Kuramitsu and Loeb (1921) have shown that female guinea-pigs spayed following delivery suckle their young quite as well as do normal animals. The effects of castration on the mammary glands did not become manifest until suckling ceased. Hammond (1925), in the rabbit, occluded the nipples on one side in lactating females and observed that rapid regression occurred in the gland on that side, although the contralateral glands continued to secrete actively. Furthermore, it is well known that cows which are not milked regularly will show a rapid decline in milk production.

The experiments of Kuramitsu and Loeb, and of Hammond have been repeated. It was found that two females spayed on the day of delivery suckled their young quite as successfully as did control females. Two females on which one nipple was occluded by ligation or adhesive tape, showed rapid regression in that gland while the control gland secreted copiously under the stimulus of suckling. Furthermore a series of females were removed from their young a few days following parturition and "milked" twice daily by hand as completely as possible. Two of

these were spayed and two served as controls. No difference was observed in the duration of lactation. Within two weeks the flow had practically ceased in all animals. This was true of the spayed animals despite the fact that removal of the ovaries has been shown to be a definite stimulus for lactation in the non-pregnant female. These observations were of especial interest because we have never been able to maintain lactation induced by pituitary extracts indefinitely even when increasing amounts of extracts were administered. Asdell (1932) has shown that administration of pituitary extract to lactating goats is effective only for a short time in preventing the decline of milk yield.

DISCUSSION

It seems to be definitely established that the mammary gland growth of pregnancy proceeds under the influence of the ovarian hormones. Turner and Frank (1932) have been able to reproduce pregnancy growth in the rabbit mammary gland by the simultaneous administration of oestrin and progestin. Although advanced mammary development may be produced in the guinea-pig by oestrin alone as has been shown by a number of workers it seems probable that the lutein hormone is concerned in the glandular development of normal pregnancy.

In the present study an analysis has been made of some of the factors which may be active in controlling the normal sequence of events occurring in the mammary gland during pregnancy and lactation. It was shown that pregnancy may continue in the guinea-pig following ovariectomy. Mammary development proceeds normally in such animals. In no instance did lactation occur until pregnancy was terminated by normal delivery or by miscarriage. It is believed that these data demonstrate the existence of a substance produced by the uterus-placenta-foetus complex which promotes mammary development and inhibits lactation. That the functional component of this complex is the placenta was shown by elimination of the other elements through various surgical procedures. There are ample reasons for believing the active principle secreted by the placenta to be oestrin.

When the entire pregnant uterus was removed, leaving the ovaries intact, lactation did not occur unless an anterior pituitary extract was administered or the ovaries were removed. This experiment seems to demonstrate that the ovaries, too, produce a hormone which inhibits lactation. However, in these animals no further mammary development occurred.

The failure of guinea-pigs to lactate following removal of the pregnant uterus by surgical procedure is not in agreement with the results of Smith and Smith (1933), who obtained lactation in rabbits hysterectomized on the 20th day of pregnancy. Loeb (1927) states that hysterectomized non-pregnant guinea-pigs show progressive mammary development which he attributes to a prolongation of corpus luteum activity induced by the operation. Although lactation did not ordinarily occur he observed

one animal ovariectomized 53 days after hysterectomy, which did lactate. As we have shown, normal non-pregnant guinea-pigs lactate following ovariectomy. We have not as yet repeated Loeb's hysterectomy experiments on non-pregnant guinea-pigs, but it is apparent from Figure 2 that in hysterectomized pregnant females the mammary glands tend to regress rather than proliferate. Although no attempts have been made to suppress lactation by preparations of the corpus luteum hormone it seems probable that the corpus luteum, too, may inhibit lactation. This would indicate that, in the guinea-pig at least, a dual check is exercised on the mammary glands during pregnancy either directly or through an action on the hypophysis.

In the earlier experiments with non-pregnant animals it was shown in males bearing functional ovarian grafts and in adult females that removal of the grafts or ovaries induced lactation. If such animals were injected with oestrin following removal of the ovarian tissue lactation could be inhibited. However, if in addition to oestrin, a potent pituitary preparation was given, lactation occurred. These experiments seemed to indicate that the ovarian hormones may inhibit lactation by suppressing the secretion of the lactation-inducing hormone by the anterior hypophysis. Selye, Collip and Thomson (1933) have reported experiments which support this concept. Rats, the mammary glands of which had been developed under the influence of ovarian stimulation induced by gonad-stimulating extracts, lactated following ovariectomy. However, if the hypophyses were removed such animals failed to lactate. Furthermore, Smith and Smith (1933) were able to inhibit lactation in parturient and pseudo-pregnant rabbits by the administration of oestrin. These various studies as well as others seem to have definitely shown that the ovaries produce one or more hormones which in some way prevent milk secretion in glands which are prepared for secretory activity. It is possible, of course, that oestrin inhibits lactation by directly influencing the mammary glands. Fellner has advanced this explanation to account for his own results and the reports of other workers. However, it is apparent that this explanation fails to take into account the established relationship of the anterior hypophysis to lactation.

A second hypothesis would accredit oestrin with the inhibition of lactation by direct action upon the mammary glands. Lactation would then be initiated by the anterior-lobe hormone acting in the absence of oestrin. This hypothesis takes into account the ample evidence for the lactation-inducing function of the anterior pituitary and is supported, for the rabbit, by the evidence of Smith and Smith, who were able to inhibit the lactation induced with pituitary extracts by the simultaneous injection of large quantities of oestrin. Some of the evidence presented here points to the same relationship in the guinea-pig, viz., that large amounts of oestrin inhibit lactation in spite of the administration of a potent pituitary preparation. However, it was shown that this relation did not hold when smaller

quantities of oestrin were given. Lactation occurred in animals injected with sufficient oestrin to inhibit lactation, if the anterior-lobe extract was administered. Lactation also occurred in hysterectomized pregnant females when the extract was injected. These experiments—together with the earlier ones (Nelson, 1932)—all indicate that oestrin may inhibit lactation through the suppression of the anterior hypophysis. It is well known from the experiments of Leonard, Meyer, and Hisaw (1931), Moore and Price (1932), and others that the gonad hormones exert a suppressing action upon the anterior hypophysis.

Moore and Price have shown that in animals the gonads of which are definitely injured by the injection of oestrin or testis hormone the administration of anterior lobe material restores the gonad functions. They have interpreted this as indicating the existence of a reciprocal relationship between the hypophysis and the gonads. It seems probable that a somewhat similar relation exists between the ovarian hormones and the anterior hypophysis in the control of lactation.

It is suggested, therefore, that during pregnancy the ovarian hormones, oestrin and progestin, promote mammary proliferation, but at the same time inhibit lactation by suppressing the secretion of the lactation-inducing hormone by the anterior hypophysis. It seems probable, too, that oestrin when it is present in large amounts, as it has been demonstrated to be in some animals during pregnancy, suppresses lactation by a direct action upon the mammary glands. At the time of parturition the oestrin level falls rapidly, the inhibitory influences acting upon the mammary glands and the hypophysis are no longer active, and lactation occurs. It seems probable that the secretion of the lactation-inducing hormone and the suspension of a direct oestrin influence upon the mammary glands are both important factors in the initiation and maintenance of normal lactation following parturition.

Consideration must be given to the fact that the mammary glands respond both to the ovarian hormones and to the lactation-inducing hormone of the hypophysis. The response to the ovarian hormones is one of growth, whereas, the pituitary hormone induces a secretory response. It is conceivable that the glands are unable to respond completely to the lactation-inducing stimulus while under the influence of growth-promoting stimuli. This would seem to be true only when the ovarian hormones are present in large amounts, as was shown in the present study. After lactation is well established the capacity of oestrin to inhibit secretion is reduced or absent (Smith and Smith, 1933). Thus it would appear that the glands are unable to respond completely to both growth and secretory stimuli at the same time. It seems probable that in the parturient female the time and quantity factors of the relationship between the gonad and pituitary hormones form a balanced and physiologically adjusted system. The precise relations between these factors must be investigated further

before they can be properly evaluated. The hypophysectomized animal should be of particular value in this regard.

The maintenance of lactation for extended periods of time seems to be partially under the control of the pituitary hormone, but a more important factor is the stimulation imposed on the secretory tissue by the continual draining of the glands in suckling. It is entirely possible, of course, that suckling may operate through the anterior hypophysis in stimulating further milk production.

SUMMARY

1. The injection, into nine pregnant guinea-pigs, of a potent anterior lobe extract terminated pregnancy and induced lactation.

2. Extirpation of the ovaries from twenty-five pregnant females did not invariably lead to abortion (nine went to term). Lactation occurred only after foetal expulsion whether abortive or at term.

3. Surgical removal of the entire pregnant uterus from twelve females did not cause lactation unless the anterior lobe extract was administered.

4. Surgical removal of the uterus and the ovaries from five females did induce lactation.

5. Surgical removal of the ovaries and the fertile uterine horn only, in four cases of unilateral pregnancy, resulted in lactation.

6. Lactation did not occur in five females when the ovaries and embryos were removed (leaving the placentae intact), during the period of placental retention, but did occur following expulsion of the placentae.

7. Lactation in ten parturient females was stimulated or prevented by varying the amounts of oestrin administered and by administering or withholding the pituitary hormone.

8. It is concluded that the ovarian hormones are active in the production of mammary gland growth during pregnancy, but that they inhibit lactation during that period. The high content of ovarian hormone in the pregnant animal appears to inhibit the secretion of the lactation-inducing hormone of the anterior hypophysis as well as to act directly upon the mammary glands. With the decline in ovarian hormone content at parturition the inhibitory influences are removed, the lactation-inducing hormone is secreted, and lactation occurs.

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TREATMENT OF SEXUAL UNDERDEVELOPMENT IN THE HUMAN MALE WITH THE ANTERIOR PITUITARY-LIKE HORMONE OF URINE OF PREGNANCY*

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Since announcements by Zondek and Aschheim (1) and Smith and Engle (2) that pituitary transplants would induce precocious sexual maturity in immature rats and mice and the discovery by Zondek and Aschheim (3) of the gonad-activating hormone in the urine of pregnant women, a wealth of experimental knowledge on the pituitary-gonadal relationship has been developed. While Engle (4, 5) and Evans and Simpson (6) have demonstrated clearly that the actions of pregnancy urine extracts are not identical with those of anterior pituitary transplants or extracts, their actions both as a luteinizing agent and as a gonad-activator so closely similates that of pituitary extracts that their use has been generally adopted. In fact, Engle has shown that the pregnancy urine extracts are possibly more effective than actual pituitary extracts in stimulating descent of the testes of monkeys. This indication is of the utmost significance because of the availability and universal use of the urine extract. While the greater part of the experimental work has been done on female animals, there is sufficient proof that the hormone from pregnant urine activates the gonads of both male and female. For this reason Bugbee (7) and associates have termed this hormone the "universal non-specific sex hormone."

In the report to follow the effect of this hormone on the development of the male genitalia is discussed. Its use in the male is founded on the experimental work on rats of Lower and Johnson (8) and of Engle (5) working with rats and monkeys, both demonstrating beyond question its stimulating effect. Riddle (9) had previously demonstrated enlargement of the testes of doves and pigeons using anterior pituitary extracts.

Campbell (10) has reported favorable results following the injection of an anterior pituitary-like hormone into women with secondary amenorrhea. Its effect on the development of the male genitalia appears to be the better method of measuring its value as a human gonad-activator, because of the ready visualization of subnormal development and the plainly evidenced effect of treatment.

PRESENT INVESTIGATION

Thirteen boys who have come under observation during the past two and one-half years, had genital underdevelopment among their chief complaints. Their ages ranged from ten to twenty-one years. Of the thirteen

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subjects studied two have been selected for rather detailed report because, before intensive treatment was begun, they were both beyond that age when normal adolescent awakening might be expected. Although Patient 2 was but fourteen years old when first seen, he had failed to respond to the treatment outlined, and genital development remained markedly retarded at the age of fifteen years nine months, when daily injections were begun.

For the most part the pregnancy urine extract used was Antuitrin-S (Parke, Davis & Co.).* Follutein (E. R. Squibb & Co.) was used in Patients 5 and 7.

Case No. I. 8-12-31. J. C., aged 18 years. The chief complaints were overweight, genital underdevelopment, short stature, and disease of the right hip joint. The patient was the second of two children, having had a normal delivery at full-term. His weight at birth was 7.7 pounds; the onset of dentition was not recalled; walking began at eighteen months; and although he spoke monosyllables and a few disyllables earlier, he did not construct sentences until four years of age. Excessive weight was present from early childhood, and at the age of six he was given "pituitary" treatment. His increase in weight had been gradual and progressive throughout life. He had been treated with thyroid and pituitary preparations, but he had never adhered very strictly to any therapeutic or dietary regime. Genital underdevelopment had caused considerable concern but it had been hoped that this would adjust itself in time. As to stature, his mother thought that his growth in the past four years had been at a complete standstill although no accurate record had been kept. The disease of the right hip began four years previously, first manifested by a limp, and upon investigation, destruction of the right femur was found. The patient had a number of orthopedic opinions, which included Perthes disease and slipping of the upper epiphysis of the femur, the latter opinion being strongly favored. He had been under the care of Dr. Willis Campbell, Orthopedist of Memphis, for treatment of the hip.

He had had the common childhood diseases. The tonsils and adenoids were removed at the age of five; appendectomy was performed at the age of sixteen.

His appetite was excessive. He was greatly dependent upon his family, his mother describing him as an "overgrown baby."

His mother was of large stature with moderate obesity. His father was of short stature. An older sister was of small physique.

A physical examination made by Dr. A. M. Thompson showed his weight to be 118.5 pounds and his height 62 inches. The optimal weight and height** for that age are 125 pounds and 66.6 inches, respectively. The blood pressure was 98.70 mm. Hg., the temperature 98.2°, and the pulse rate 72.

There was a rather pronounced general adiposity with localized deposits over the mammae and over the mons veneris. No facial, axillary, or pubic hair growth was noted. The underdevelopment of the genitalia was striking. The right testicle was about the size of a grapefruit seed, and was localized in the inguinal canal; the left testicle could not be palpated. The prostate was not defined. There was impairment of gait; the right leg was shorter than the left and there was atrophy of its muscles. Limitation of motion of the right hip joint was noted.

Laboratory Findings. A single specimen of urine was slightly amber in color and clear. The specific gravity was 1.020. There was an acid reaction. There was no albumin nor sugar in the urine. Microscopically there were many mucous threads, occasional epithelial and white blood cells; many colon bacilli were present. The blood hemoglobin was 76 per cent of normal; white blood cells numbered 10,050; the red blood cells, 4,450,000. Polymorphonuclears constituted 61 per cent, small lymphocytes 26 per cent, large lymphocytes 6 per cent, eosinophiles 3 per cent, basophiles 1 per cent and transitionals 3 per cent of the differential count. A Wassermann test was negative. Blood calcium was 10 mgm. per 100 cc. of blood (normal 9-11 mgm.). The basal metabolic rate was minus 5 per cent (average of repeated tests).

*Acknowledgment to Dr. E. A. Sharpe, Parke, Davis & Co., for a plentiful supply of Antuitrin-S is hereby made.

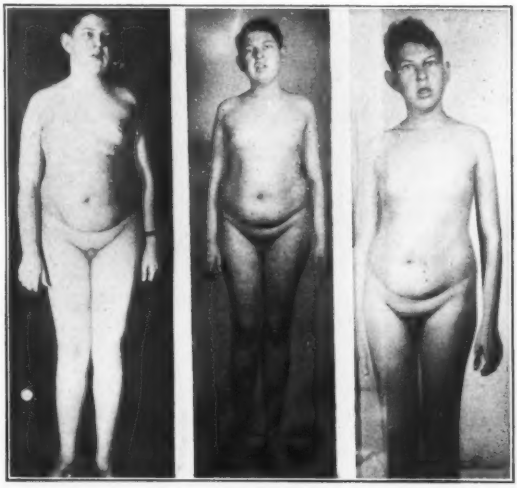
**Optimal weight and measurement standards throughout this paper are adopted from Endocrine Medicine, Wm. Engelbach, Chas. C. Thomas Co., Springfield, Ill., and Baltimore, Md., 1932.

Roentgenograms. The sella turcica appeared to be normal. Studies of the wrist, hand, foot, elbow and pelvis revealed a marked delay toward fusion with development corresponding to chronological age of about twelve years. In the right hip there was destruction of the entire head of the femur. Slight scoliosis to the left in the lower lumbar region was noted.

Diagnosis. (1) Adiposo-genital pituitarism with secondary hypothyroidism; (2) bilateral cryptorchism; right testicle palpated in inguinal canal, left not defined; (3) slipping of the upper right femoral epiphysis; left scoliosis in lower lumbar area; (4) bacilluria.

Treatment. (1) Desiccated thyroid, one grain three times daily; (2) Antuitrin, 2 cc. intramuscularly three times weekly; (3) Pituitrin to tolerance intramuscularly three times weekly; (4) anti-obesity diet; (5) treatment for bacilluria consisting of Hemitol, five grains three times a day after food, and free use of water; (6) continuation of orthopedic measures previously instituted.

Progress. (1-1-32.) The patient objected to hypodermics and did not take them with any regularity, likewise he was inconsistent with thyroid medication. No improvement was noted in his physical condition. Beginning January 1, 1932, Antuitrin-S, 100 units, and Antuitrin, 1 cc. were given three times weekly without interruption.



A B C

Case No. 1. A. Aged 18 yrs. Genital aplasia; bi-lateral cryptorchism. B. Four months later after Antuitrin-S., 300 u. daily. Both testicles well defined in scrotum. C. Eight mos. later after Antuitrin-S, 200 u., and Antuitrin (No. 128), 1 cc. daily. Further enlargement of testicles, increase in size of penis and first appearance of pubic hair.

Re-examination. (2-2-32.) The weight was 125 pounds and the height 62¼ inches. There was no change at this time with the exception of an increase in weight. There was a suggestion of the left testicle being palpable in the canal. The pulse rate was 78 and the blood pressure 100/70. The temperature was 98.2°.

Laboratory Findings. The urine examination gave negative findings. The blood morphology findings were normal. The blood calcium was 9.6 mgm. per 100 cc. of blood (normal 9-11 mgm.). The basal metabolic rate determination was minus 10 per cent.

Roentgenograms. Studies of the wrist, hand, and elbow joints in both views failed to show any appreciable advancement toward fusion of the epiphyseal centers.

An ocular examination by Dr. J. N. McGrath gave negative findings except for a slight general contraction of form fields in both eyes.

An orthopedic opinion by Dr. A. Horwitz considered the condition of the right hip to be that of Perthes's disease, while Dr. M. L. Klinefelter considered the condition to be that of slipping of the upper femoral epiphysis.

Treatment. (1) Antuitrin-S, 300 units daily; (2) desiccated thyroid, two grains daily; (3) anti-obesity diet. This treatment was continued daily without interruption until June 6, 1932 (four months).

Re-examination. (6-6-32.) The weight was 92½ pounds, and the height 62¼ inches. This was a loss of 27½ pounds in weight, but there was no change in stature. The pulse rate was 90 and the blood pressure 88/68. The genitalia showed limited, though definite, improvement, both testicles had descended into the scrotum, and both were about the size of a large pea. There was less complaint from the patient about the hip condition, and motion was somewhat less limited.

Laboratory Findings. The urine examination gave negative findings. The blood morphology findings were normal. The basal metabolic rate was plus 4 per cent.

Roentgenologic examination of the wrist and hand and lateral ankle showed slight advancement toward fusion since the last examination. The condition of the right hip had not changed since the previous examination.

Treatment. (1) Antuitrin-S was reduced to 200 units daily and 1 cc. of Antuitrin was given daily; (2) 1 grain of desiccated thyroid was given three times a day and (3) 2 gelatin capsules of Haliver oil upon retiring; (4) the diet was less restricted.

Further examination (10-17-32) disclosed that the treatment prescribed four months before had been followed without interruption. The weight was now 97 pounds and the height 63¼ inches, an increase of one inch. There was definite increase in the size of the external genitalia; the scrotum showed pronounced enlargement, and both testicles were distinctly enlarged, being the size of hazel nuts. There was also some pubic hair growth. The pulse rate was 96, and the blood pressure 96/52.

Treatment. (1) Antuitrin-S, 200 units daily; (2) Antuitrin, 1 cc. daily; (3) desiccated thyroid, 3 grains daily; (4) Haliver oil, 2 gelatin capsules on retiring; (5) calcium lactate in a glass of milk three times daily; (6) limitation of diet.

About February 20, 1933, the treatment was interrupted by an orthopedic operation on the right hip.

Discussion. The striking points in the physical examination were genital aplasia and statural underdevelopment, showing a lack of both the gonad-stimulating and the growth-promoting hormones of the anterior pituitary. X-ray studies revealed a marked delay in closure of the epiphyseal centers, a finding long associated with advanced hypopituitarism. That an hypophyseal disturbance was suspected in early childhood is ascertained from the history that pituitary treatment was administered at the age of five years. The favorable reaction to thyroid medication verified the diagnosis of secondary hypothyroidism.

At the age of eighteen years and four months, when intensive treatment was begun, the possibilities of further genital development without outside stimulation were nil. Only one testicle, the right, could be defined. This was located in the inguinal canal and was markedly undersized. Undoubtedly the left testicle was in the canal but it could not be differentiated with any certainty from other soft parts.

After four months' treatment with Antuitrin-S, 300 units daily injected into the gluteus, both testicles were palpated in the scrotum. They were small, each the size of a large pea. During this time there had been no statural growth, and advancement toward fusion of the epiphyseal centers was slight. In the next four and one-half months there was treatment with Antuitrin-S, 200 units, and Antuitrin, 1 cc. daily. The testicles

increased to the size of hazel nuts with accompanying enlargement of the scrotum, although the penis was not appreciably changed in size. While genital development in this lad remains definitely subnormal, descent of the testicles with an increase in their size is considered a positive result in view of the initial hopelessness of spontaneous improvement.

Case No. II, 2-21-31. C. W., aged 14 years. The chief complaints were genital underdevelopment and overweight. The patient was the first of four children. His weight at birth was six and one-half pounds. Dentition was said to have occurred at six to seven months. He began walking and talking between twelve and thirteen months.

Genital underdevelopment alarmed the parents about two years ago, prompting them to consult a urologist. Both testicles having been found to be undescended, a bi-lateral operation was performed, but the hoped-for development of the external genitalia and the testicles following this operation was not realized. The patient was reported as behaving normally in every way—he played hard, was alert generally, and did well in school work. He had always had a nervous temperament and had lost some time in school because of this nervousness. He had an excessive appetite, being particularly fond of sweets, and had been more or less overweight throughout his entire life. No medical or dietary treatment had been followed for obesity.

Past and personal histories disclosed no relevant abnormalities.

One sister, aged 10, showed evidence of early adolescence. One maternal uncle, aged 26, had been treated for genital underdevelopment; this uncle is six feet three inches in height.

PHYSICAL EXAMINATION

	Patient's	*Optimal*
Weight	147.75 lbs.	89 lbs.
Height	64.37 ins.	59.8 ins.
Span	68 "	61.1 "
Upper Measurement	28.12 "	29.5 "
Lower Measurement	36.25 "	30.3 "
Bust	37 "	28.6 "

The measurements indicated eunuchoidism and the patient was definitely of the eunuchoid type. There was moderate general adiposity about the trunk, the pectoral girdle being especially large. The skin and hair were of normal texture. The patient's perception and response were good. The temperature was 99.6. Blood pressure in the right arm was 138/66, and in the left arm, 120/76. The pulse rate was 84.

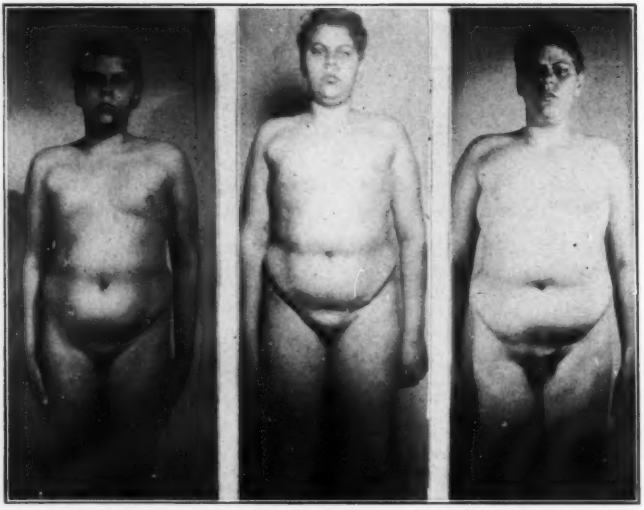
There was a scar just above and parallel to the inguinal ligaments on both sides. The external genitalia showed marked underdevelopment. The scrotal sac was found to be very small, adhering closely to the perineum. The testicles were palpated in the inguinal canals, being determined only by local thickenings. There was no pubic or axillary hair present. On rectal examination the prostate could not be defined.

Laboratory Findings. A single specimen of urine had a yellow color, was clear, and had a specific gravity of 1.010; the reaction was acid and there was no albumin or sugar present; microscopical examination showed some mucous threads and occasional white blood cells. The blood hemoglobin was 80 per cent of normal; the white blood cells numbered 7,500 and red blood cells, 4,400,000. Differential count showed band forms, 3 per cent; segmented forms, 63 per cent; small lymphocytes, 31 per cent; monocytes, 1 per cent; transitionals, 1 per cent, and eosinophiles, 1 per cent. The Wassermann test was negative. The blood calcium was 9.6 mgm. per 100 cc. of blood. The fasting blood sugar level was 86.9 mgm. per 100 cc. One hour after dextrose it was 96.6 mgm. and two hours after it was 88.5 mgm. The blood non-protein nitrogen was 28.7 mgm. The basal metabolic rate determination was minus 5 per cent.

Rooentgenologic examination of the skull (for sella turcica) and of the chest gave normal findings. The osseous system showed slightly advanced development.

Treatment. (1) Antuitrin-S, 100 units three times weekly; (2) restriction of diet.

Progress. The treatment was carried out for a period of ten months. The testicles had increased in size to about that of a lima bean and could be definitely located, although they remained high in the inguinal canals. For the next nine months, no treatment was administered, but it was resumed at the end of that period. The weight at this time was 166.5 pounds, and the height 66 inches; upper measurement, 29.5 inches; lower measurement 36.7 inches; and span 68.5 inches. Some slight advancement in development of the genitalia had occurred, manifested by an increase in the size of the testicles, although they remained high in the inguinal canals. Injections of Antuitrin-S, averaging 300 units each were given on an average of five days out of each week. There resulted a remarkable increase in the size of the testicles, until they corresponded in size to a hickory nut. They came to lie in the pubic fat tissue just outside of the scrotum, which remained small and closely adherent to the perineum. The penis increased proportionately in size, and pubic and axillary hair became abundant. No evidence of nocturnal emissions or other genital function has been observed to date.



A B C

Case No. 2. A. Aged 14 yrs. Genital aplasia. Unsuccessful operation for undescended testicles. B. Nineteen mos. later. For first 10 mos. Antuitrin-S, 300 u. weekly. No treatment in past 9 mos. Very slight improvement. Testicles remained high in canals. C. Two years later after Antuitrin-S, 1500 u. weekly for 5 mos. Testicles low in canals and enlarged. Penis enlarged. Scrotum small. Pubic and axillary hair growth.

The diagnosis of eunuchoidism based on genital aplasia and marked overdevelopment of long bones seems justifiable in this case. Neither testicle was descended before treatment was instituted. After ten months' treatment with Antuitrin-S, 100 units three times a week, the size of the testicles was slightly increased. When the patient was fifteen years nine months of age and intensive treatment was begun the testicles enlarged to a moderate size and descended to the scrotal sac, the penis enlarged, and pubic and axillary hair growth occurred.

Cases 3 to 13 (Chart Nos. I and II) comprise a group of eleven boys with varying degrees of genital underdevelopment. Nine of the eleven responded favorably to treatment. It may be of importance to note that all of these nine were obese types. While the two non-obese types (Cases 12

CHART I

Case No.	Age in Years	Weight	Height	Measurements			B. M. R. %	X-Ray		Laboratory				
				Upper	Lower	Span		Sella	Osseous Develop.	Hb (Sahli)	WBC	Bl. S.	NPN	Wass.
								Chest		RBC	Smear	S. Tol.	Cal.	Urine
1 J. C.	18	118.5 *125.0	62.0 66.6	29.4 32.8	32.6 33.8	63.2 68.7	− 5	N	Delayed Fusion	76% N	10,050 N	N	 N	N N
2 C. W.	14	147.7 *89.0	64.3 59.8	28.1 29.5	36.2 30.3	68.0 61.1	− 5	N N	Slightly Advanced	N N	N N	N N	N N	N Col. B.
3 A. S.	10	113.2 *64.9	58.3 53.2	28.3 26.7	30.0 26.5	59.0 53.6	+ 3	N	N	74% N	N 42% L			N Tr. Alb.
4 J. V.	11	121.5 *75 0	60.5 56.2	27.5 27.9	32.0 28.3	62.5 56.9	+10	N	N	N Subnormal	N N			N N
5 E. T.	11.6	148.7 *75.4	62.0 56.2	28.2 27.9	33.7 28.3	64.5 56.9	− 6	N	Slightly advanced	N N	N N			N Tr. Alb. Gran. C.
6 J. W.	12	141.7 *78.7	60.7 57.1	29.2 28.3	31.5 28.8	62.0 58.0	−14	N	N					N N
7 E. M.	13	142.0 *85.4	60.5 56.2	27.2 29.1	33.3 29.8	61.7 60.1	− 3 −12	N	Slightly advanced	N N	N N		 N	N N
8 J. S.	13	149.5 *85.4	65.2 58.9	30.7 29.1	34.5 29.8	66.0 60.1	− 8	N	Slightly advanced	N N	N N	72.2	N N	N N
7 R. S.	13	121.0 *85.4	61.0 58.9	29.7 29.1	31.2 29.8	60.5 60.1	− 2 −17	N N	N	N N	N 38% L	N	N N	N N
10 R. G.	13.4	125.5 *89.0	61.0 59.8	....	....	....	− 5	N	N	N N	N 49% L			N N
11 P. Mc.	13.5	101.0 *89.0	56.8 59.8	27.8 29.5	30.0 30.3	58.5 61.1	−16 − 9	N	N	N N	N 45% L 39% L	N	N N	N N
12 W. T.	16	118.0 *109.0	64.2 64.0	29.2 31.5	35.0 32.5	71.0 65.8	− 9 −17	N	Delayed Fusion	N N	N N	N	39.3 N	N N
13 L. K.	21	123.5 *135.0	69.2 68.0	32.2 33.7	37.0 34.3	71.5 70.4	+14							N N

*Optimal for age.**
**Endocrine medicine—Wm. Engelbach, Chas. C. Thomas, Springfield and Baltimore, Vol. I, Chap. VII. N = Normal. L = Lymphocytes.

and 13) did not react satisfactorily, this may well be attributed to inadequate treatment as was well demonstrated in Subject 2, who responded with intensive treatment after less active measures had failed.

A review of the measurements of these lads is of interest. Except for Case 11, the height was greater than optimal for the given age. The lower measurement exceeded the upper and the span was greater than the height in each instance. This would indicate an excess of the growth hormone in the presence of insufficient sex hormone.

CHART II

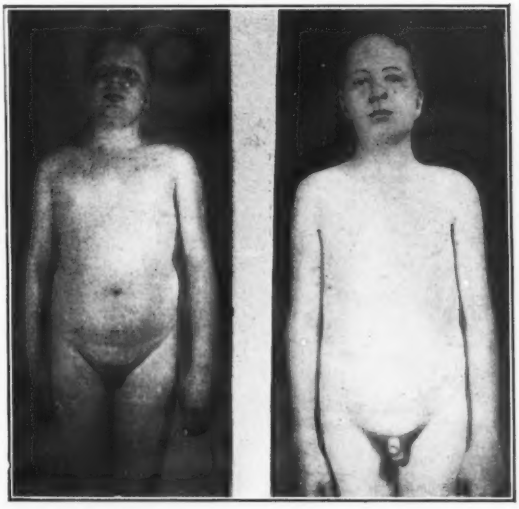
Case No.	Age in Years	Genital Development	Treatment	Genital Response
1 J. C.	18	Marked aplasia. Rt. tes. in canal, size of navy bean. Lt. tes. not localized. P—undefined.	As-300 u daily for 4 mos., 7 days a wk. As-200 u and Ant. 1 cc. daily for 4 mos.	Both tes. descended. Size of large peas. Further enlargement of tes. and scrotum.
2 C. W.	14	Marked aplasia. Bi-lat. cryptorchism. Tes. high up in canals. P—undefined.	As-100 u 3 X wk. for 10 mos. No treatment for 9 mos. As-1500 u wkly. for 5 mos.	Position of tes. unchanged; slightly increased in size. Unaltered. Tes. definitely larger. Not completely descended, but low in canals. Penis larger. P—undefined.
3 A. S.	10	Lt. cryptorchism. Rt. tes. high in scrotum. P—undefined.	As-100 u 2 X wk. for 4 mos.	Lt. tes. descended. Both tes. low in scrotum, subnormal in size. P—undefined.
4 J. V.	11	Rt. tes. undescended. Lt. tes. subnormal size. P—undefined.	As-50 u 2 X wk. for 11 mos.	Lt. tes. normal size. Rt. tes. in scrotum; very small size. P—barely outlined.
5 E. T.	11.6	Subnormal. P—undefined.	F-25 u and Pit. 2 X wk. for 4 mos.	Normal development. P—defined.
6 J. W.	12	Marked aplasia. Bi-lat. cryptorchism. Rt. tes. in canal. Lt. tes. not defined. P—undefined.	6/7/30 Ant. 1 cc. and Thyr. 12/30/30 As-50 to 100 u 3 X wk. 10/22/31 As-50 u 2-3 X wk.	12/12/30 No improvement 4/11/31 Rt. tes. descended 2/13/32 Both tes. descended Normal development. P—defined.
7 E. M.	13	Markedly subnormal. P—questionably defined.	F-50 u 3 X wk. for 6 mos.	Normal development. P—defined.
8 J. S.	13	Markedly subnormal. P—undefined.	As-100 u daily for 6 days a week.	After 6 mos. definite improvement, but still subnormal size. P—undefined.
9 R. S.	13	Subnormal. P—outlined.	As-50 u and Pit. 2 X wk. Thyroid for 5 mos.	Normal development. P—slightly larger.
10 R. G.	13.4	Subnormal. P—outlined.	As-50 u and Pit. 2 X wk. for 6 mos.	Normal development. P—larger.
11 P. Mc.	13.5	Rt. cryptorchism. Lt. tes. and penis subnormal. P—undefined.	4/10/31 As-100 u 2 X wk. and Des. Thyroid. 1/5/33 Above continued.	4/10/31 Limited improvement. Rt. tes. undescended. 1/5/33 Further improvement. Rt. tes. descended. P—defined.
12 W. T.	16	Markedly subnormal. P—defined.	Ant. 1 cc. and As-100 u 3 X wk. for 10 mos. Thyroid.	Only slight improvement. P—no change.
13 L. K.	21	Tes. very small. Penis normal. Eunuchoid type. P—well defined.	As-150 u 2 X wk. for 4 mos.	No change. P—no change.

As=Antuitrin—S; F=Follutein; Pit=Pituitrin; Ant.=Antuitrin; P=Prostate; u=units.

Cryptorchism was present in Subjects 3, 4, 6, and 11. The left testicle in Subject 3 remained ectopic over the pubis, but increased in size. Complete descent of the testicles resulted in Subjects 4, 6, and 11. In Subject 4 the left testicle developed fully while the right remained small. In Subject 6 development of both primary and secondary sex characteristics was normal, while in Subject 11, genital development in general was retarded to a slight degree.

Aside from delayed epiphyseal fusion in Subject 12, osseous development was normal throughout the series.

The lowest basal metabolic rate recorded was minus 17 per cent, in Subjects 9 and 12. In Subjects 6, 7, and 11 the basal metabolic rates were below minus 10 per cent. All five of these boys were given desiccated thyroid with a favorable response in every instance.



A B

Case No. 4. A. Aged 11 yrs. Rt. cryptorchism. Operated for this 1 yr. previously. B. Eleven mos. later after Antuitrin-S, 100 u. weekly. Rt. testicle in scrotum but small. Lt. tetsticle well developed.

A study of the blood showed a tendency to lymphocytosis in Subjects 3, 9, 10, and 11; otherwise the laboratory findings were essentially within normal limits.

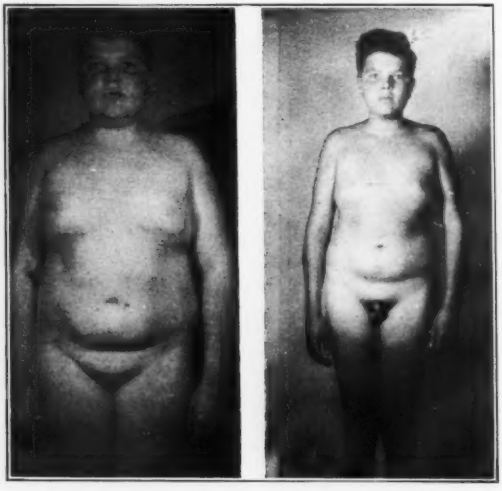
GENERAL DISCUSSION

In several cases (3, 4, 5, 7, and 9) there possibly was an equal chance of normal development occurring without any treatment whatever, for this delayed adolescent awakening has often been observed in patients of this type.

Recent work by Engle (5) gives evidence that the pronounced increase in weight and size of the testicles in immature rats and mice produced by either the injection of pregnancy urine or of pituitary trans-

plants is not true precocious maturity since there is no acceleration of gametogenesis. In the cases treated no evidence was obtained which pointed to early maturity, although an accurate study was not made. The size of the prostate corresponded to the size of the external genitalia. Where underdevelopment of the latter was pronounced, the prostate could not be definitely defined. With increase in size of the genitalia, there occurred a corresponding increase in the size of the prostate.

The high incidence of cryptorchism in genital underdevelopment is shown by its presence in six of the thirteen cases. While no conclusions are advanced from such a small number, the finding of this many cases is striking and may be attributed to the faultiness of these undescended organs. Of the six subjects, two had been operated upon (Cases 2 and 4). In Subject 2, in whom both testicles were identified in the canals and re-



A B

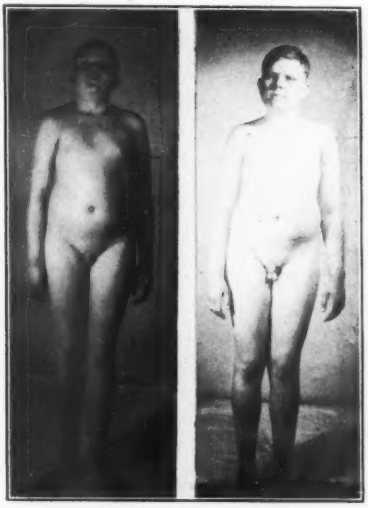
Case No. 6. A. Aged 12 yrs. 6 mos. Genital aplasia. Bi-lateral cryptorchism. B. Eight mos. later after Antuitrin-S, 100 u. weekly. Both testicles descended. Genital development only slightly subnormal for age.

mained there well beyond adolescence and even after intensive treatment was begun, hope for their spontaneous descent could hardly have been entertained. In Subject 4, no proof is offered that proper descent would not have taken place without treatment in due time, but the descent was probably hastened by the treatment.

In the cases of cryptorchism in which operation was not performed, proper descent without treatment had certainly failed in Subject 1. Even with this boy at the advanced age of eighteen, both testicles entered the scrotum after treatment. The left testicle in Subject 3 increased in size but remained in its ectopic position over the pubis. Considerable doubt is held that proper descent and development would have occurred spon-

taneously in Subject 6. It was only after two years' constant treatment that both testicles were located in the scrotum. Had treatment been more intensive in this case, the time, no doubt, would have been shortened. The fourth instance of cryptorchism without operation was found in Subject 11. Again prolonged treatment was necessary to bring both testicles into the scrotum.

These clinical data on undescended testicles coincide with Engle's experimental work on the macaque monkey. Since the human testicles normally are descended at birth, it may be assumed that an ample supply of gonad-activating hormone is furnished the foetus through its own pituitary or by that of the mother or possibly, as advanced by Engle (5), from the hormone present in the maternal circulation during pregnancy. The gubernaculum testis is evidently not the only factor in bringing about



A B

Case No. 8. A. Aged 13 yrs. Marked genital hypoplasia. Both testicles descended. B. Four and one-half mos. later after Antuitrin-S, 100 u. daily. Genital development closely approaches normal for age.

descent of the testes, for an adequate supply of the pituitary sex hormone seems essential.

From the clinical evidence presented, underdevelopment of the genitalia in the boy can be corrected with injections of anterior pituitary-like extracts if sufficient treatment is instituted before the completion of adolescence. Large doses and frequent injections are more likely to bring about the desired results, especially if the boy is near the end of the adolescent period. If younger, doses of 100 units two to three times weekly appear adequate, as nearly as can be judged from the small number studied. In boys beyond fifteen years of age, the chances for complete

correction are diminished. Certainly at this age treatment must be intensive.

When cryptorchism exists, whether an operation has or has not been performed, it would seem advisable to institute this treatment because of the faulty condition of most undescended testicles. In this manner the chances of failure would be greatly diminished.

SUMMARY

Thirteen boys with genital underdevelopment of varying degrees and ranging in age from ten to twenty-one years were treated with intramuscular injections of the anterior pituitary-like hormone of pregnancy urine. Eleven of the subjects responded to treatment by an increase in the size of the external genitalia and the appearance of secondary sex characteristics. These eleven were obese, while the two who failed to respond were thin. The treatment instituted in these two cases was limited. Cryptorchism was present in six of the thirteen cases, and in four of these six the testicles localized in the scrotum after treatment. In one, that of a boy eighteen years of age, the right testicle was the size of a grapefruit seed localized high in the inguinal canal, while the left testicle was undefined. After daily injections of 300 units over a period of four months both testicles were well defined in the scrotum. After continued daily injections for another four months' period the testicles developed to the size of hazel nuts and a sprinkling of pubic hair appeared for the first time. Another boy with testicles in the canals that were differentiated only by local thickening was fifteen years nine months old, when injections of 300 units, five days a week, were begun. After six months the testicles had increased to the size of hickory nuts and lay just out of the scrotum. Pubic and axillary hair, not present before, had appeared.

The dosage and duration of treatment was dependent on the degree of underdevelopment and the age, those in early adolescence responding most favorably.

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THE HISTOLOGICAL FINDINGS OF THE HYPOPHYSIS IN CANCER*

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Although the literature affords a mass of information concerning deviations in the histologic structure of the anterior lobe of the hypophysis, but little has been published regarding histological findings in this structure in cases of malignant tumors, especially carcinomata. These variations appear with a certain regularity and a fixed morphological picture, showing corresponding changes associated with hypofunction of the other endocrine organs (Berblinger).

Thus Karlefors (14), as well as Berblinger and Muth (2), examined the anterior lobe of the hypophysis in cases of carcinoma with widespread metastasis. In 14 male subjects ranging from 18 to 60 years of age on whom autopsy had been performed, Karlefors was able to point out that the "*Hauptzellen*" played the chief rôle; also in four of five cases of sarcoma. A similar condition obtained in 14 of 17 cases of women suffering from carcinoma, as well as in one woman who died of sarcoma. From their own material, consisting of 30 males (24 of whom died of carcinoma, four of sarcoma and two of myeloma) and 13 females who died of carcinoma and one of ovarian sarcoma, Berblinger and Muth (2) have confirmed these findings of Karlefors. These authors in their histological findings, laid their principal stress upon results found in male cases, since in women previous pregnancies influence the cell picture of the anterior lobe in the sense of the known pregnancy changes through enlargement and increased number of Hauptzellen. On the basis of their findings, Berblinger and Muth (2) came to the conclusion "that in about 50 per cent of all cancer deaths, these Hauptzellen will be found with the same frequency in male subjects as in female subjects." These authors, agreeing with Karlefors, emphasized, however, "that the increase of Hauptzellen at no time attains the extent reached in pregnancy and that the hypophyseal picture in carcinoma differs widely from that of pregnancy." Schmincke (26), too, was able to show a proliferation of Hauptzellen in the case of a 19-year-old virgin with bilateral, secondary ovarian carcinoma. Saenger (25) found a like condition in a 55-year-old woman whose right ovary had been removed two and three-fourth years prior to her death. In the left ovary a metastasis was found which had destroyed the organ. In addition to abnormal lactation of the mammae an extraordinary proliferation of Hauptzellen was found in the enlarged, tumor-like hypophysis.

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Kraus (16) studied 13 cases of carcinoma as to the incidence of basophile cells; in five extremely emaciated subjects they were somewhat strikingly diminished. Contrariwise, in one case, that of a strong, fairly well nourished man who had died of pulmonary carcinoma, they were greatly increased. In the remaining cases normal or very slightly altered values were found irrespective of the degree of cachexia. In six cases of cachexia resulting from diseases other than carcinoma, he reports either normal, slightly increased or decreased values, the latter in two cases with distinctly reduced suprarenals.

Kiyono (15) was able to demonstrate in carcinoma cases six instances of Hauptzellen, six instances of eosinophile and three instances of basophile increase. Wohlwill (31) was unable in any way to confirm a constant shifting in the quantitative relation of cells of the anterior pituitary in carcinomatous subjects.

The work so far referred to, primarily devoted to the confirmation of the morphological findings of the anterior lobe, led to an attempt to explain them. In his findings Berblinger thought he had to do with the action of foreign blood proteins, just as he was inclined to account for a similar change in the hypophysis in hypothyroidism by a disturbance of the albumin metabolism, an opinion which he more recently (1932) has been inclined to discard in order to avoid further attempts towards explanation.

More recent research in relation to hormones (Aschheim and Zondek, et al.) has again directed particular attention to the histopathological findings of the anterior lobe, the purpose being, if possible, to determine histologically the local cells in which the various hormones were produced. In this connection Berblinger (1) emphasized that in female genital carcinoma the reaction for anterior pituitary hormone is frequently positive while, on the contrary, it rarely proves positive with the urine of women with benign genital neoplasms. Zondek, explaining the results of his investigations, is of the opinion that the pituitary reaction is positive in 25 per cent of women with benign genital tumors and 81 per cent in patients with malignant genital tumors. He advances the possibility that through the growing tumor, the anterior lobe of the hypophysis will stimulate a greater formation of ripe follicular hormones which are then stored in the tumor cells. According to Gostimirovic (12), Prolan A, which appears in the urine in increased quantities in cancer cases, is produced in the anterior hypophysis. Proceeding from the findings of Zondek (32), Hofbauer (13) discusses in two communications the relationship of the anterior hypophysis to uterine carcinoma and supports his opinion upon his own animal experiments in which, following the injection of hypophyseal extract and the implantation of anterior lobe substance in guinea pigs, hyperplastic endometritis with cystic change of the ovaries was observed. Accordingly, this author perceives in a hyperfunction of the hypophysis one of the factors which produces endometrial hyperplasia and at the same time proliferations of the vaginal epithelium of the experimental animals.

TABLE I

Weights of Hypophyses—Our Material

Age	Sex	Diagnosis	Weight
66	Female	Ca. Breast	1.305 gm.
64	Male	Ca. Stomach	0.655 gm.
51	Male	Ca. Prostate	0.605 gm.
46	Female	Ca. Rectum	0.905 gm.
70	Female	Ca. Stomach	0.450 gm.
52	Male	Ca. Stomach	0.680 gm.
62	Female	Ca. Rectum	0.710 gm.
60	Female	Ca. Gallbladder	0.775 gm.
62	Female	Ca. Breast	0.820 gm.
75	Female	Ca. Gallbladder	0.452 gm.
63	Male	Ca. Stomach	0.698 gm.
58	Female	Ca. Stomach	0.800 gm.
58	Female	Ca. Gallbladder	0.825 gm.
67	Female	Ca. Liver	0.600 gm.
59	Female	Ca. Stomach	0.795 gm.
61	Female	Ca. Breast	0.980 gm
57	Female	Ca. Cervix	1.200 gm.
54	Male	Ca. Bronchus	1.000 gm.
50	Male	Ca. Stomach	1.150 gm.
56	Female	Ca. Liver	0.920 gm.
81	Male	Ca. Esophagus	1.000 gm.
34	Female	Ca. Uterus	1.100 gm.
45	Female	Ca. Intestine following Wertheim	1.005 gm.
35	Female	Ca. Ethmoid Plate	1.505 gm.
63	Male	Ca. Stomach	1.005 gm.
71	Male	Ca. Bladder	0.860 gm.
60	Male	Ca. Esophagus	0.752 gm.
45	Female	Ca. Maxilla	0.762 gm.
85	Female	Melano-Ca	0.850 gm.
61	Male	Ca. Pancreas	0.765 gm.
71	Female	Ca. Esophagus	0.825 gm.
50	Male	Ca. Bronchus	0.540 gm.
53	Male	Ca. Esophagus	0.950 gm.
65	Female	Ca. Breast	0.910 gm.
80	Male	Ca. Lung	0.785 gm.
54	Female	Ca. Gallbladder	0.700 gm.
80	Male	Ca. Prostate	1.025 gm.
62	Female	Adamantinoma	0.670 gm.
74	Male	Ca. Stomach	1.175 gm.
45	Male	Ca. Bronchus	0.550 gm.
51	Male	Ca. Bronchus	0.850 gm.
55	Female	Ca. Liver	0.850 gm.
48	Female	Following Wertheim	1.065 gm.
72	Female	Ca. Vulva	0.875 gm.
71	Male	Ca. Rectum	0.885 gm.
54	Female	Ca. Bladder	0.925 gm.
33	Female	Ca. Breast	0.725 gm.
78	Male	Ca. Stomach	0.800 gm.
62	Male	Ca. Right Colon	0.825 gm.
58	Female	Ca. Stomach	0.650 gm.
38	Male	Ca. Stomach	0.790 gm.
42	Female	Ca. Sigmoid	0.940 gm.
75	Female	Ca. Maxilla	0.725 gm.
70	Male	Ca. Esophagus	0.800 gm.
68	Female	Ca. Vulva	0.725 gm.
62	Male	Ca. Breast	0.575 gm.
54	Male	Ca. Esophagus	0.725 gm.
43	Female	Ca. Uterus	0.850 gm.
55	Male	Ca. Bronchus	0 650 gm.
58	Male	Ca. Rectum	0.600 gm.
46	Female	Ca. Breast	0.830 gm.
58	Female	Ca. Stomach and Uterus	0.810 gm.
79	Female	Ca. Right Colon	0.920 gm.
55	Male	Ca. Esophagus	0.720 gm.
61	Male	Ca. Esophagus	0.900 gm.
77	Male	Ca. Sigmoid	0.750 gm.
53	Male	Ca. Rectum	0.700 gm.
32	Female	Branchiogenetic Ca	0.875 gm.
57	Male	Ca. Bronchus	1.350 gm.
61	Male	Ca. Stomach	0.675 gm.
52	Female	Ca. Bronchus	1.120 gm.
66	Male	Ca. Rectum	0.640 gm.
74	Female	Ca. Breast	0.880 gm.
67	Male	Ca. Tongue	0.470 gm.
55	Male	Ca. Bronchus	0.900 gm.
36	Male	Ca. Penis	0.550 gm.
70	Male	Ca. Pancreas	0.650 gm.
54	Male	Ca. Rectum	0.785 gm.
56	Male	Ca. Lung	0.740 gm.
64	Female	Ca. Bladder	0.550 gm.

This latter conclusion gives Hofbauer occasion to raise the question of the origin of the epithelial proliferations in the human cervix.

Upon histological examination of human material in 30 cases of malignant tumors, Susman (29) referred in a further English work to pathological findings in the hypophysis. As to the significance of the rôle of the hypophysis in the etiology of carcinoma, he states that these cases presented an increase of the eosinophiles which he regards as indicating a hyperfunction of the anterior lobe. He infers that the hardening of the posterior lobe, which he found in 21 of his cases, came from a hypofunction of this part. Examinations of the pancreas in 14 cases disclosed in 12 an enlargement of the isles of Langerhans.

The examinations of Engle (8) concerning the relations between cancer and pituitary follow a similar course. In his experiments a positive Aschheim-Zondek reaction occurred in all cases in which the experimental animals had received three or more subcutaneous injections of urine from cancer subjects. On the other hand, the Aschheim-Zondek reaction proved negative in all patients suffering from sarcoma. Upon these findings Engel concludes that between hyperfunction of the hypophysis and carcinomatous growth there exists a causal connection.

In the available literature concerning the morphological changes of the hypophysis in cancer, no uniformity of opinion exists as to the quantitative combination of cells. Interested in the study of the hypophysis in cancer cases, I proposed to Prof. Anders that the wealth of material in the Pathological Department of the Rudolf Virchow Krankenhaus in Berlin be studied along these lines, under his direction. Our material comprised 80 cases of carcinoma of different organs from patients of various ages. For comparison with established findings we undertook a large number of control examinations. For legal reasons the hypophyses could not be removed until 24 hours after death. After fixing in formalin, weighing and imbedding in paraffin, the histological examination followed from sections cut in a sagital direction in order to show the anterior lobe, posterior lobe, hypophyseal stalk and pars tuberalis. For staining, in addition to the usual haemotoxin-eosin method, we also employed the Mallory method in some cases, besides the special staining method introduced by Kraus for the purpose of more positively differentiating the individual cell forms of the anterior lobe.

MATERIAL

Concerning the *average weight* of the hypophysis, numerous tables have been compiled according to sex [Rasmussen (23), Petersilie (21), Simmonds (27), Erdheim-Stumme (7)] which Berblinger (1) assembled and arranged in comparative weight values with his own findings in 2442 cases. For the purpose of better comparison these numbers are given so far as they have meaning for our material:

Average normal values, *male*, different ages:

Age	Rasmussen	Petersilie	Simmonds	Erdheim-Stumme	Berblinger
30–40	0.575	0.628	0.741	0.643	0.649
41–50	0.560	0.634	0.687	0.614	0.656
51–60	0.472	0.600	0.666	0.600	0.642
61–70	0.505	0.634	0.660	0.612	0.617
71–80	.....	0.633	0.697	.....	0.606
81–90	.....	.....	0.599	.....	0.586

Average normal values, *female*, different ages:

30–40	.....	.....	0.814	0.746	0.711
41–50	.....	.....	0.775	0.689	0.715
51–60	.....	.....	0.798	0.755	0.733
61–70	.....	.....	0.754	0.757	0.731
71–80	.....	.....	0.730	.....	0.707
81–90	.....	.....	0.755	0.715	0.700

The established weights of the hypophyses in our 80 cases are entered in Table I. For consideration of the different ages and both sexes, the corresponding values are taken from this table and placed in Tables II and III.

TABLE II

MALE

Age		Average
30–40	0.790 (38)—0.550 (36)	0.670
41–50	1.150 (50)—0.540 (50)—0.550 (45)	0.746
51–60	0.605 (51)—0.850 (51)—0.680 (52)—0.950 (53)—0.700 (53) 0.785 (54)—1.000 (54)—0.725 (54)—0.650 (55)—0.720 (55) 0.900 (55)—0.740 (56)—1.350 (57)—0.600 (58)—0.752 (60)	0.800
61–70	0.765 (61)—0.675 (61)—0.900 (61)—0.825 (62)—0.575 (62) 1.005 (63)—0.698 (63)—0.655 (64)—0.640 (66)—0.470 (67) 0.800 (70)—0.650 (70)	0.721
71–80	0.885 (71)—0.860 (71)—1.175 (74)—0.750 (77) 0.800 (78)—0.785 (80)—1.025 (80)	0.897
81–90	1.000 (81)	1.000

TABLE III

FEMALE

Age		Average
30–40	0.875 (32)—0.725 (33)—1.100 (34)—1.500 (35)	1.050
41–50	0.940 (42)—0.850 (43)—1.005 (45)—0.762 (45)—0.905 (46) 0.830 (46)—1.065 (48)	0.908
51–60	1.120 (52)—0.700 (54)—0.925 (54)—0.850 (55)—0.920 (56) 1.200 (57)—0.800 (58)—0.825 (58)—0.650 (58)—0.810 (58) 0.795 (59)—0.775 (60)	0.864
61–70	0.980 (61)—0.710 (62)—0.820 (62)—0.670 (62)—0.550 (64) 0.910 (65)—1.305 (66)—0.600 (67)—0.725 (68)—0.450 (70)	0.772
71–80	0.825 (71)—0.875 (72)—0.880 (74)—0.452 (75)—0.725 (75) 0.920 (79)	0.779
81–90	0.850 (85)	0.850

When one calculates the average weight of all 80 cases from Tables II and III, the following is shown:

TABLE IV

Age	Male	Female
30–40	0.670	1.050
41–50	0.746	0.908
51–60	0.800	0.864
61–70	0.721	0.772
71–80	0.897	0.779
81–90	1.000	0.850

The average weights of the hypophysis in the carcinoma cases examined by us are higher in the different age classes than the normal weights computed by the above-named authorities. Tables V and VI show the established weights of the hypophysis in both sexes in carcinoma cases of the different organ-systems:

TABLE V

MALE

Age	Genitourinary Tract	Alimentary Tract	Respiratory Tract
30–40	0.550 (36)	0.790 (38)	
41–50		1.150 (50)	0.550 (45)—0.540 (50)
51–60	0.605 (51)	0.680 (52)—0.950 (53) 0.700 (53)—0.725 (54) 0.785 (54)—0.720 (55) 0.600 (58)—0.752 (60)	0.850 (51)—1.000 (54) 0.650 (55)—0.900 (55) 0.740 (56)—1.350 (57)
61–70		0.900 (61)—0.675 (61) 0.825 (62)—0.698 (63) 1.005 (63)—0.655 (64) 0.640 (66)—0.470 (67) 0.800 (70)	
71–80	0.860 (71)—1.025 (80)	0.885 (71)—1.175 (74) 0.750 (77)—0.800 (78)	0.785 (80)
81–90		1.000 (81)	
	Average, 0.760	Average, 0.797	Average, 0.818

TABLE VI

FEMALE

Age	Genitourinary Tract	Alimentary Tract	Respiratory Tract
30–40	1.100 (34)		0.875 (32)—1.500 (35)
41–50	0.850 (43)—1.005 (45) 1.065 (48)	0.940 (46)—0.905 (46)	
51–60	0.925 (54)—1.200 (57)	0.800 (58)—0.650 (58) 0.810 (58)—0.795 (59)	1.120 (52)
61–70	0.550 (64)—0.725 (68)	0.710 (62)—0.450 (70)	
71–80	0.875 (72)	0.825 (71)—0.920 (79)	
81–90			
	Average, 0.921	Average, 0.780	Average, 1.165

In comparing the average weights of the hypophysis in carcinoma assembled in Table IV with the above-mentioned normal values, one sees that they are considerably greater for the men as well as for the women in all age classes in carcinoma cases. Between 51 and 60 the average weight of the male hypophysis as estimated in the tables of the different authorities is 0.596 gm., while that of the carcinoma cases is 0.800 gm. The difference in weight appears just as clearly in females; for instance, between 41 and 50 the average weight is 0.726 gm., while in carcinoma cases it is 0.908 gm. Between 51 and 60 the organ has an average weight of 0.762 gm., while in carcinoma cases it is 0.864 gm.

The comparative weights of the hypophyses in carcinoma of the different organ-systems in both sexes are presented in Tables V and VI. It is clear that carcinomata of certain organ-systems are especially characteristic of one or the other sex. While in carcinoma of the genitourinary tract the average value of the male hypophysis is 0.760 gm., that of the female genitourinary tract is 0.921 gm. In carcinoma of the alimentary tract the weight differences are not so evident in the two sexes: in contrast to the average weight of 0.760 gm. in women is a corresponding value of 0.797 gm. in men.

When we review the hypophyseal weights in our 80 cases it appears that in a considerable percentage in which autopsy revealed a general cachexia, the hypophysis not only did not participate in the cachetic atrophy but increased in weight, so that in the seventh to the eighth decade the male hypophyseal weights almost reached 0.9 gm.

DISCUSSION

The question was, therefore, how is this apparent paradox in the weight increase of the hypophysis in carcinoma cases to be explained. Theoretically, there was the possibility that the weight increase resulted from local foreign-cell material as, for example, metastasis of the primary tumor. In fact, our histological examinations proved that in both cases in which we were able to observe the maximum weight of the hypophysis of 1.505 gm. and 1.305 gm., a marked carcinomatous infiltration of the organ was present. These cases follow:

No. I. S. N., 110-33. Name, M. T. Age, 35; female; height, 162 cm.; weight, 47 kgm.

Clinical Diagnosis. Condition of patient following operation: Carcinoma of the ethmoid plate extending over the orbit, the sphenoid and the antrum.

Anatomical Findings. Principal disease—Carcinoma of accessory cavities of the nose. Contributory cause of death—Paralysis through infiltration of tumor into the cerebrum.

Diagnosis. Condition following enucleation of right bulbus oculi: Filling of both sides of ethmoid plate, sphenoid and right antrum and part of right orbit, with carcinomatous tissue; destruction of bone substance. Considerable destruction of the base of the skull in the region of the right orbital roof, the cribiform plate of the ethmoid and the right pyramidal apex. Further extension of carcinoma into the right frontal lobe of cerebrum. Extensive destruction of the sella turcica. Hypophysis macroscopically free from tumor tissue (weight, 1.505 gm.). Further metastasis of liver and portal lymph glands. The bony system is free from metastatic foci. Marked anemia of all organs.

(Signed) ANDERS.

No. II. S. N., 1223-32. Name, A. L. Age, 66; female; height, 153 cm.; weight, 48 kgm.

Clinical Diagnosis. Mammary carcinoma with metastasis. Thrombosis of the superficial veins of the left lower leg.

Anatomical Findings. Principal disease—Carcinoma of left breast, general metastasis. Cause of death—Tumor anemia and cachexia.

Diagnosis. Carcinoma *en cuirasse* of left breast with axillary lymph gland metastasis; severe bilateral carcinomatous pleurisy with hemorrhagic exudate; intrapulmonary carcinomatous nodules in the right lung. Metastasis in roof of skull as well as head of right femur and in broncho-pulmonary lymph glands. Flabby, anemic and dilated heart. Moderate arteriosclerosis of aorta and coronary vessels of heart. Relative enlargement of hypophysis (weight, 1.305 gm.). Marked anemia and brown atrophy of all organs.

(Signed) ANDERS.

In the first case, in addition to the pars anterior, the pars posterior was involved in an extensive carcinomatous infiltration. In the second case a metastasis permeated the entire pars intermedia, pars posterior, and part of the pars tuberalis. Clinically, it is striking that these patients during their lifetime had never shown any evidence of hypophyseal insufficiency, although in the second case the normal relationships between the compressed anterior and posterior lobes in relation to the infundibulum, were almost completely displaced.

Aside from the foregoing cases of maximum hypophyseal weight, in only three others of our 80 cases was metastasis histologically evident, a ratio of 6.2 per cent. According to Simmonds (28), Neubürger (20) and Kiyono (15), carcinoma of the breast metastasizes relatively frequently in the hypophysis. In our material only one such case appeared (Case 2 above). The other three cases mentioned were a carcinoma of the bladder in a 54-year-old woman, a rectal carcinoma in a 71-year-old man and an esophageal carcinoma in a 60-year-old man. In spite of carcinomatous tissue in these three cases the weights of the hypophyses were less than the average weights of the hypophyses in carcinoma of the different organ-systems of both sexes. To be sure, these cases concerned only microscopically proven metastases.

For a second cause of the increased weight of the hypophysis we bore in mind the possibility that the squamous epithelial masses described by Erdheim (6) displayed signs of growth in advancing age (Kraus) in especially large measure in the region of the hypophyseal stalk. Among our 80 cases these epithelial islands were found in 15 cases, or 18¾ per cent; however, in none was their quantity so great that they could have been the cause of the increased weight. Histologically, the epithelial masses showed no proliferative appearance and they could always be differentiated without difficulty from metastatic carcinoma.

After determining that neither the Erdheim cells nor metastatic carcinoma foci could be the cause of the increased weights, we were obliged to search for other causative factors. We had in mind the overfilling of the capillaries due to passive congestion but a review of our preparations proved that this, too, had not attained a degree which could have produced the increased weight.

Thus there remained as a final possibility the fact that the parenchyma of the hypophysis itself, through the increase in size of one or the other of its parts, brought about the weight increase of the organ as a whole. Since the cause could only be proliferative change of the local cells, it was necessary to bear in mind the parts of the hypophysis which came into the question, namely, the pars anterior, pars intermedia and pars tuberalis. According to our findings the two latter parts of the organ showed an entire absence of change. It therefore remained to examine the different cells of the pars anterior for their reciprocal numerical ratios.

Familiar with the previous results of examinations concerning the histological changes in the hypophysis in carcinoma cases, we tried at first to picture the normal quantitative shiftings of the various cells through physiological age changes, directing our special attention to the eosinophile and basophile cells. In agreement with Kraus (16, 17), Cooper (4) and others, we noted that beginning about the fiftieth year there is a continually increasing reduction in the number of the eosinophiles and also that the individual cells become smaller. They show in part "indistinct cell margins and lie here and there imbedded in thin, faintly-colored masses which have possibly arisen from disintegrated, non-granular cells" (Kraus, (19). The basophiles, according to Cooper (4) display a proportionate increase in advancing age. We found them especially numerous in the region of the anterior pole and the peripheral zone towards the pars intermedia, while the eosinophiles occupied the central part of the lobe (Bell, 3; Vandenburg, 30, and Fraser, 11). These basophils frequently exhibited in advancing age a strong accumulation of vacuoles which, upon staining, proved to be enveloped with a fine covering of fat. If we still add that the interstitial connective tissue of the pars anterior experiences a certain increase and hyaline swelling with age, we have mentioned the phenomena which should be considered with regard to the age changes of the hypophysis.

As to the *functional significance* of the basophile cells, the latest findings of Zondek (34) and Fels (10) assert that the chief sexual hormone is produced by them. On the basis of these newer findings Zondek, in agreement with Evans and Simpson (9) and Berblinger (1), claimed the basophile cells produced Prolan, while Philipp (22) in 1930 regarded the eosinophile cells as the seat of its production. Besides the production of follicular hormones the basophile cells have, according to Kraus (18), further important relations to the fat and cholesterin metabolism and to the regulation of blood pressure.

Regarding the hormonal functions of the eosinophiles, clinical observations in connection with experimental examinations have led to the result that they are to be regarded as the place of production of the growth hormone (Berblinger, 1; Cushing, 5, et al.). According to Kraus (18), on

the basis of his observations in cases of acromegaly and diabetes mellitus, one is warranted in accepting the fact that it is the eosinophile cells which are truly responsible for the effect upon the carbohydrate metabolism.

In turning to the results of our histological examination concerning the numerical relation of the basophile and eosinophile in carcinoma, it was seen in the beginning that no fixed laws were to be observed. With due respect to the fact cited by Kraus (18) that the basophiles show an increase in individuals with constant high blood pressure, especially associated with primary and secondary contracted kidney, in the beginning of our examinations we did not consider all cases in which autopsy had disclosed this condition as a secondary finding accompanying carcinoma. We hoped to obtain as far as possible pure cases but met with some difficulty since the hypertension in connection with severe athero-arteriosclerosis played a rather large part in the material at our disposal.

Collectively, the histological examinations in pure cases of carcinoma, that is, not complicated with severe arteriosclerosis or hypertension, yielded the following: In these cases a quantitative increase of the eosinophiles was established which, beginning apparently in the ventral part of the anterior lobe, developed in the direction of the pars intermedia, so that in especially striking cases the central part of the anterior lobe consisted almost entirely of eosinophile cells. The single cells often were distinguished by large size and their distinct affinity for eosin. In contrast to these proliferated appearances of the eosinophiles we were unable to establish a quantitative increase of Hauptzellen. In some cases it was remarkable that the basophiles located in the peripheral part of the anterior lobe showed an enlargement. It formed such a picture as Kraus (16) has described and shown as a compensation hypertrophy of the basophiles, for example, at the border zone of an eosinophile adenoma. In the remainder of these pure cases of carcinoma the basophiles showed neither increase nor decrease. In the protoplasm of these cases there appeared frequently the aforementioned larger or smaller vacuoles. The plasma of these cells was often conspicuous by a particularly high grade basophilia.

In comparing these findings of uncomplicated carcinoma cases with numerous complicated cases, in which autopsy had proved the existence of a more or less severe arteriosclerosis or in which the organ changes indicated hypertension, we see that there was also the described quantitative increase of eosinophiles, except that here the basophiles had experienced a simultaneous increase. In many cases one had the impression that the basophiles displayed a certain enlargement. In this connection the fact is noteworthy that in many of these cases, in spite of very severe carcinomatous metastasis throughout the body, no tumor cachexia existed; on the contrary, autopsy revealed an especial adiposity. In some of these cases the cortex of the adrenals showed a particular abundance of lipoids.

If we examine the communicated macroscopical and histological findings critically, it appears that the above-mentioned weight increase of the organ is explained by the histological examination; that there are proliferative processes especially of the eosinophile apparatus to which by the introduction of arteriosclerosis or hypertension is added proliferation of basophile cells, representing in their totality the cause of the weight increase.

Consequently, the results of our examination in regard to the changes of the quantitative composition of the anterior lobe cells agrees with those which Susman (29) set up from his carcinoma cases. The quantitative increase of the eosinophiles was for this author the expression of hyperfunction of the anterior hypophysis. Like Kraus, we were able to establish that the basophiles experience a relative increase in severe atherosclerosis and hypertension, even though a carcinoma existed at the same time.

It would seem that in spite of the constancy of these findings and in spite of the increase of a safe knowledge concerning the function of the different cell forms of the anterior lobe, a certain reservation is demanded. If, in the course of a carcinoma, a quantitative cell picture appears in the anterior lobe—as we know happens in castration in the sense of increased eosinophiles—we should first point to the fact that the eosinophile cells are to be regarded as the place of production of the growth hormone and that a proliferation of these cells, for instance, in an adenoma or an already pure hyperplasia, leads to particular growth signs, as in acromegaly. In none of our cases was acromegaly or acromegaloid growth revealed, hence the next question appeared to be whether an increased production of the growth hormone runs parallel to the proliferations of the eosinophile cells in carcinoma cases. As to a normal or pathological growth-stimulus in the internal organs or the extremities, we have discovered no findings relating to this, so that it seems doubtful whether the exuberant growth of eosinophiles of the anterior lobe in cases of carcinoma produces normal growth hormone in increased quantity. On comparing the values of the eosinophile increase with the degree of metastatic development in the different cases it appeared that a proliferation of the eosinophile cells is not parallel to the degree of metastatic formation. On the ground of our histological findings we can at this time render no definite conclusions regarding the functional meaning of the proliferation of eosinophile cells in carcinoma cases.

SUMMARY

1. In 80 cases of carcinoma of different organs and in different ages it was established that in 6.2 per cent the hypophysis was the seat of metastasis. Preferential inclination of breast carcinoma to metastasize in the hypophysis could not be observed.

2. Of the hypophyses in the 80 cases the average weight in both sexes and in all age classes was increased. Histologically the increase involved the cells of the anterior lobe, especially the eosinophile cells.

3. The functional meaning of the proliferation of the eosinophile cells is not evident; no tendency to acromegaloid changes could be detected.

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THE CHEMICAL NATURE OF EMMENIN

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Early in 1930, we (1, 2) reported the discovery in acetone extracts of human placentae of a substance which induced the phenomena of oestrus in intact immature rats in doses much smaller than those required for adult ovariectomized rats, which was active by oral route, and which was not extracted from acid aqueous solutions by ether. At that time the only substance known to be capable of inducing oestrus in ovariectomized rodents was the theelin or ketohydroxy-oestrin isolated the year before by Doisy and by Butenandt. This substance was known to be active in adult ovariectomized rats in doses certainly no larger than those required for immature intact rats; it was considered to be only feebly active by oral route, and it was not known to occur in ether-insoluble complexes. We therefore felt justified in giving to our placental fraction a new name, emmenin, and supposed its high activity in the immature intact rat to be due to stimulation of the ovary.

In 1930, however, Marrian (3) isolated a second member of the oestrin series, the so-called theelol or trihydroxy-oestrin. Although at first this new substance appeared to possess fairly high activity as assayed in ovariectomized animals (4, 5), it became apparent that the ratio between the minimal dose for the ovariectomized adult and the minimal dose for the intact immature rodent was much larger than in the case of ketohydroxy-oestrin (4) though still smaller than in the case of emmenin. It appeared also that the oral activity of the substances of this series had been seriously underestimated (4). Moreover, evidence began to appear that in some circumstances, for example in the urine of pregnant mares (6, 7, 8), these substances might occur in ether-insoluble form, presumably as esters (hydrolyzed by acid) or otherwise combined with some unknown water-soluble material. It was clear that the distinctions upon which we had relied in separating emmenin from the oestrin series were no longer water-tight. We had also consistently failed to obtain histological evidence that emmenin had any stimulating effect upon the ovaries.

Further light upon the question was shed by the discovery and isolation (9) from the ether-soluble fraction of the placental extracts of a crystalline subtance which had the chemical properties of trihydroxy-oestrin (theelol) but the characteristic physiological properties of emmenin. The total amount required to produce a cornified vaginal smear in intact rats 21 days old, receiving six injections in 36 hours, was about 0.0013 mg., while at least 0.015 mg. was required to produce a similar reaction, by

similar technique, in adult castrate rats. A sample of theelol, kindly supplied by Doctor Doisy, had the same potency as our crystals when tested on immature intact rats, but was active in adult ovariectomized rats in doses as small as 0.0015 mg. However, Butenandt and Stormer (10) reported that the high and variable activity of trihydroxy-oestrin in adult ovariectomized rodents was due to the presence of small amounts of the keto-hydroxy form; when this contaminant was completely removed, the activity fell to 75,000 mouse units per gram. MacCorquodale, Thayer and Doisy (11) were unable to detect any such contamination in samples of "theelol"; it would seem, nevertheless, that some of their earlier preparations, such as the one examined by us (9) and referred to above, may have contained sufficient ketohydroxy-oestrin to explain their high activity in ovariectomized animals. Butenandt and Browne (12) compared a recent preparation from Doctor Doisy's laboratory with a specimen of trihydroxy-oestrin carefully freed from ketohydroxy-oestrin, and concluded that they were identical in chemical composition, physical properties such as melting point and optical activity, and physiological potency: in each case, a dose of 0.01 mg. sufficed to produce oestrus in adult ovariectomized mice, when administered in oily solution in one single injection. This potency is of the same order of magnitude as that now accepted by Doctor Doisy and his collaborators. Butenandt and Browne (12) also compared with these substances a sample of the crystals obtained by us (9) from the ether-soluble fraction of the placental extracts, and concluded that all three substances were probably identical, physiologically and chemically, though the placental crystals in that particular sample were somewhat less pure and contained traces of ketohydroxy-oestrin. Doctor Butenandt kindly sent us a sample of his specially purified trihydroxy-oestrin, which we found to possess the same high activity in the immature intact rat as theelol and the placental crystals. We agree that the three substances referred to are in all probability identical.

The fact that these substances display high activity in the immature intact rodent and low activity in the adult ovariectomized animal is of very great interest. Ketohydroxy-oestrin (theelin) does not show this property at all. The fact that in the one case the test-animals are large, in the other small, is not of much significance; since it has been shown (13) that immature ovariectomized rats require much larger doses of placental or other trihydroxy-oestrin preparations than immature intact rats, or immature ovariectomized rats bearing ovarian transplants. It has also been shown (13) that the amount of oestrin secreted by the immature ovary (14, 15) probably does not influence the use of such animals for assay and cannot explain the relatively refractory condition of the ovariectomized immature animal. We have accordingly (9, 13) set up the hypothesis that these various trihydroxy-oestrin preparations have little or no direct action upon the uterus and vagina, but that they are converted by the ovary into a substance (possibly a ketohydroxy-oestrin) to

which the uterus and vagina are highly responsive. Direct evidence of such activation is at present lacking; but that the ovary is in some way concerned in the reaction of the intact immature animal is beyond question.

We conclude, then, not only that the crystals from the ether-soluble fraction of the placental extract are trihydroxy-oestrin and identical with the similar substances separated by Marrian, Butenandt, and Doisy from pregnancy urine, but also that in the original ether-insoluble emmenin fraction we are dealing with a complex of this same substance with some unknown and probably inert material. We base this conclusion not merely on the resemblance in physiological properties between the crystals and the emmenin extract, but also on the observation (9) that it is possible by autoclaving with dilute acetic acid to render the active principle of the emmenin fraction ether-soluble. Presumably under these conditions hydrolytic decomposition of the postulated complex occurs and the trihydroxy-oestrin is set free. We have, in fact, been able to separate crystals from such autoclaved emmenin fractions which, as far as our examination has gone, appear to be identical with the crystals from the ether-soluble fraction discussed above.

In earlier publications (9, 13) we have referred to the crystalline material from the ether-soluble fraction as "crystalline emmenin," because of its physiological resemblance to our original emmenin fraction. But since this material has been shown to our satisfaction to be trihydroxy-oestrin, we are now prepared to discard this use of the term, waiving all question of the priority of the name "emmenin" over the names "theelol" and "trihydroxy-oestrin." We propose, however, to retain the name "emmenin" for the ether-insoluble complex originally studied. It is highly probable that the altered solubility may make possible physiological differentiation of the complex emmenin from the liberated trihydroxy-oestrin which is its active principle; Girard (16) has recently drawn attention to the rôle which solubility plays in determining the physiological properties of substances of this type. In our clinical studies (17) the name emmenin refers always to the ether-insoluble fraction. We feel that, at least until the nature of the complex present is completely elucidated, this fraction deserves a specific name. It will be clear from the foregoing discussion that we regard as the salient physiological property of emmenin the powerful oestrogenic effect in the intact animal and the relative inertness of the material in ovariectomized animals. The results of the clinical use of this fraction may easily be reconciled with these experimental findings, and with our hypothesis that the active principle undergoes chemical change with enhancement of potency in the presence even of immature or atrophic ovaries. That actual stimulation of the ovaries occurs is a theory which we have long since abandoned, although we find in recent discussions (18, 19) that emmenin is still referred to as ovary-stimulating, and is even equated with "Prolan A"—an identification which is immediately disproved by the inactivity of emmenin in the male.

We are indebted to Doctor Doisy and Doctor Butenandt for their friendly co-operation, especially in supplying us with specimens of their crystalline preparations.

SUMMARY

The name emmenin has been given to an alcohol-soluble, ether-insoluble substance present in acetone extracts of human placentae, active orally, and powerfully oestrogenic in immature rodents with intact ovaries though relatively inert in ovariectomized animals. Crystals subsequently isolated from the ether-soluble fraction show similar physiological properties and are regarded as the active principle of emmenin. When other laboratories succeeded in preparing trihydroxy-oestrin (theelol) free from ketohydroxy-oestrin (theelin), the former was found to be identical with the placental crystals. Emmenin is therefore regarded as a hydrolysable complex containing trihydroxy-oestrin; it does not appear to be ovary-stimulating and it is suggested that its active principle is converted to some more potent form in the presence of ovarian tissue.

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GLUCOSE TOLERANCE STUDIES IN CHILDREN AND IN ADOLESCENTS*

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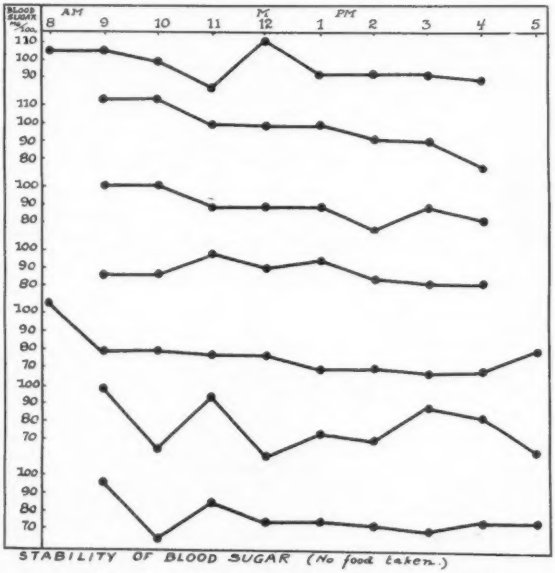
At the outset, let us refresh our minds about some of the basic factors to be considered in blood sugar studies. The normal fasting blood sugar level fluctuates between 70 and 110 mg. per cent. This level, if followed for a period of hours, fluctuates but little in the adult, as can be seen from Chart 1. This is also true of a child, and in Chart 2, I have reproduced



1. Stability of blood sugar during day time in adults.

from the splendid publication by Svensgaard, a series of blood sugar determinations on infants, aged two to five months. These show but slight fluctuation in the blood sugar levels. The second curve in the series, in which the blood sugar level rose more than in the others is the only exception, but when the blood sugars were studied the second day on this same infant, a fairly even curve resulted. These studies show that the blood sugar level in children does not vary appreciably from that in adults, and

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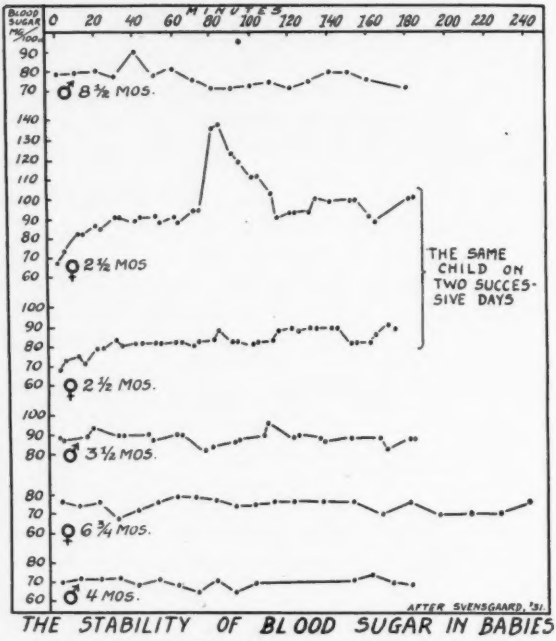
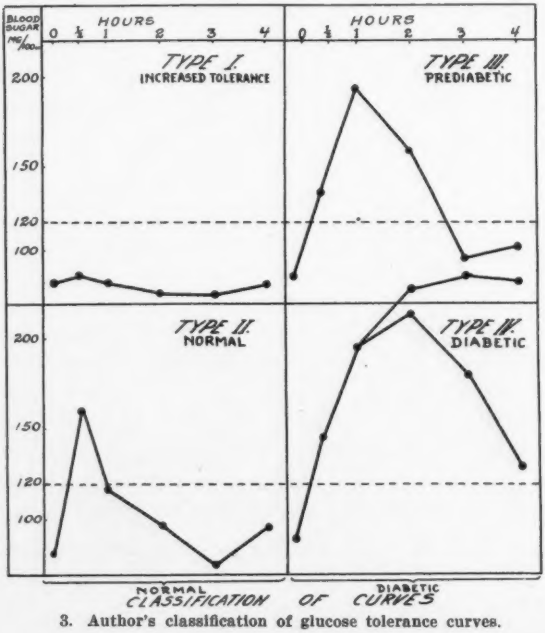

BLOOD SUGAR MG/100
MINUTES
0 20 40 60 80 100 120 140 160 180 200 220 240
♂ 8½ MOS.
♀ 2½ MOS
THE SAME CHILD ON TWO SUCCESSIVE DAYS
♀ 2½ MOS.
♂ 3½ MOS.
♀ 6¾ MOS.
♂ 4 MOS.
AFTER SVENSGAARD, '31.
THE STABILITY OF BLOOD SUGAR IN BABIES

2. Stability of blood sugar in infants.



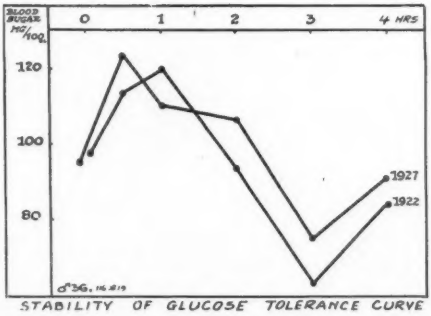
3. Author's classification of glucose tolerance curves.

we may say that the fasting blood sugar level is, for the most part, quite a uniform straight line. The values for the fasting blood sugar in the newborn and infants tend, however, to be a bit lower than in adults.

When a person partakes of food, there is a postprandial rise of blood sugar and this rise varies considerably in different individuals. This rise has been studied by many workers all over the world and has been used as a diagnostic criterion in diabetes. The test has been standardized by giving to a fasting person 100 gm. of glucose dissolved in ice cold water, or to a child 50 to 75 grams according to body weight (1.75 gm. per kg.) and then studying the extent of the rise of the blood sugar and the length of time this elevation persists. A high and prolonged rise of blood sugar is interpreted as inability of the patient to utilize the carbohydrate. In order to classify the various responses, each worker has established certain standards. Chart 3 illustrates the classification which I use:

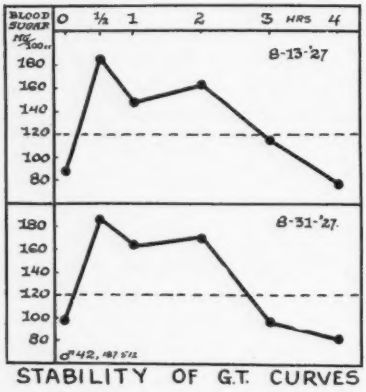
1. Very little or no rise, interpreted as increased tolerance.
2. A rapid rise and equally rapid fall (one to one and one-half hours), interpreted as normal tolerance.
3. A slower and higher rise and a slower fall (two and one-half to three and one-half hours), interpreted as decreased tolerance, a prediabetic type of curve.
4. A slow, high rise and equally slow descent (three and one-half hours or more), interpreted as a diabetic type of curve.

For further simplication Types 1 and 2 are classed as normal and Types 3 and 4 as diabetic.

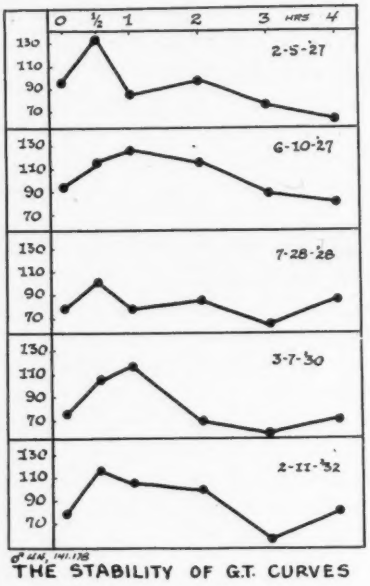


4. Stability of glucose tolerance curves in adult male. Two curves 5 years apart.

These curves, if secured under normal conditions, are quite stable and can be reproduced repeatedly on the same patient. Chart 4 represents two curves on the same patient five years apart, and they are practically identical. Even certain characteristics, such as a dip (in Chart 5) at the end of the first hour, will reproduce themselves. This I have seen often. In Chart 6 are presented five glucose tolerance curves on the same person over a period of five years and all are virtually the same.

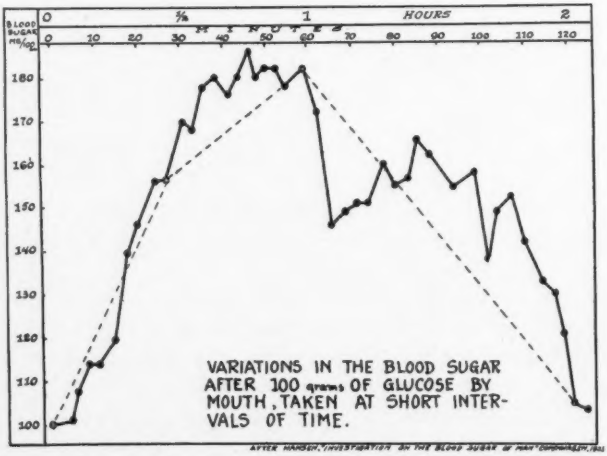


5. Stability of glucose tolerance curves in adults and reproduction in each of the characteristic "dip" at the first hour.



6. Stability of glucose tolerance curves in an adult over a period of 5 years.

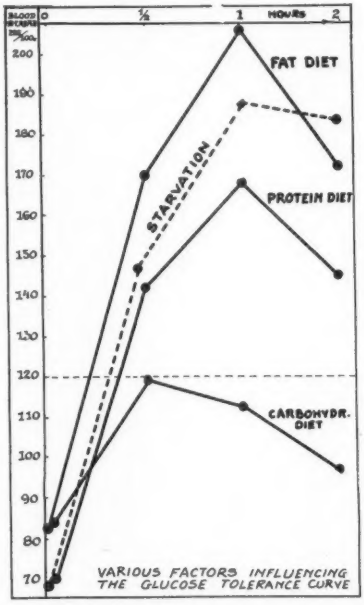
If, instead of determining the blood sugar hourly the blood sugar is estimated every ten minutes during the test, as Hansen has shown in Chart 7, the result is not a smooth curve, but a zigzag course with minor variations, the contour of which, however, corresponds closely to the hourly curve. In some it varies more; in others, less.


0 ½ 1 HOURS 2
BLOOD SUGAR
VARIATIONS IN THE BLOOD SUGAR AFTER 100 grams OF GLUCOSE BY MOUTH, TAKEN AT SHORT INTERVALS OF TIME.

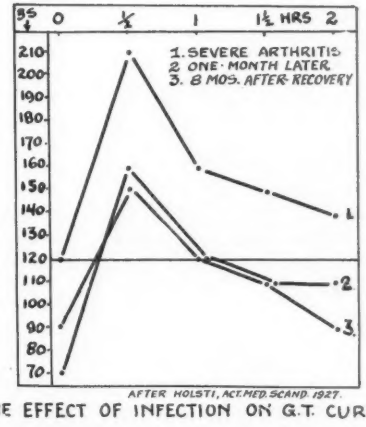
7. Glucose tolerance curve in an adult (blood sugars taken every 10 minutes), showing fluctuations when blood sugars are taken at short intervals. (Hansen's figures.)

There are certain pitfalls in interpreting glucose tolerance curves which must be avoided. Some of these have been illustrated in Chart 8. The lower curve represents a test made under normal conditions; that is, with the patient taking a normal diet. Starvation and various freak diets distort the curve, and hence the test must be carried out only under normal conditions, while the patient is leading a normal life and eating normally. This will obviate many faulty diagnoses and opinions.

There are other factors which influence the glucose tolerance curve. A patient with liver disease who is unable to store glycogen in the liver shows a diabetic type of curve. In jaundiced patients, therefore, the test is of no value. Infections also raise the curve as is shown in Chart 9; the



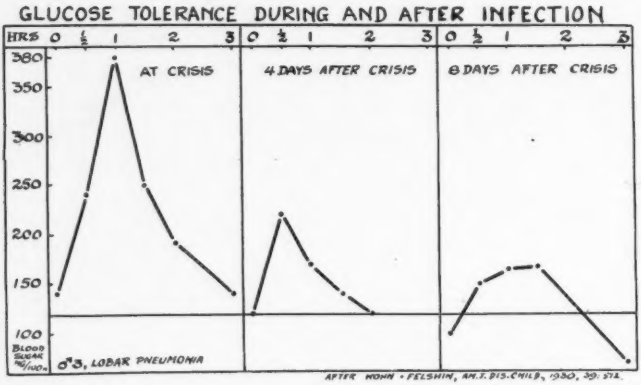
8. Various factors influencing the glucose tolerance curve.



9. The effect of infection (arthritis) on glucose tolerance curves.

first curve was made while the patient had severe arthritis, the other two, after recovery. In Chart 10, the first curve was made during a crisis of lobar pneumonia in a child aged three years and shows the abnormal rise of blood sugar; the other two were made after the infection had subsided.

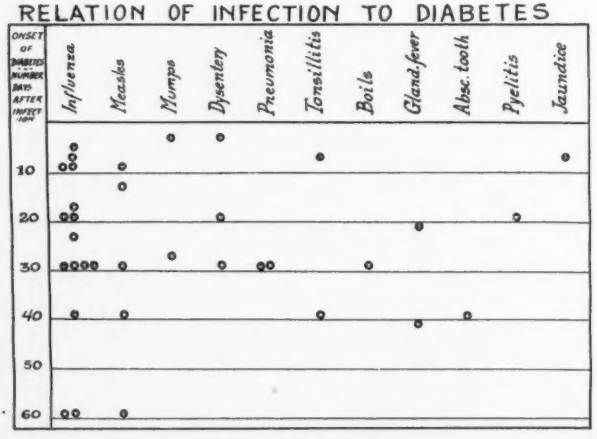
These findings during infections are not without significance and I feel that at such times there is an actual affection of the pancreas, a temporarily diminished insulogenic function. At times this process goes on to produce a true diabetic state. Chart 11 shows the short period of days intervening between various infections and the onset of diabetes in a group of children. Permanent diabetes results often in children shortly after



10. The effect of infection (lobar pneumonia) on the glucose tolerance curve in a child three years of age.

influenza, measles, mumps, dysentery and other infectious diseases, and this possibility should always be considered, in cases of childhood infections.

In the present study I have attempted to analyze a series of 192 glucose tolerance tests in children, aged four to 20 years, and to group them and classify them according to the various affections. (Table 1 presents these data in detail.) The curves are classified as indicated elsewhere (Chart 3). In Chart 12 the results of this study in the principal groups are illustrated graphically. In the groups of young patients with hyperpituitarism and rheumatic disease, there is the highest incidence of the diabetic type of curve. In cases of hyperpituitarism, there is not a frank diabetic condition, but with an imbalance of internal secretion, hyperglycemia is produced. In patients with decreased pituitary function, at the



11. The rôle which infection plays in the production of diabetes in children.

192 GLUCOSE TOLERANCE TESTS IN PATIENTS, AGED FOUR TO TWENTY YEARS

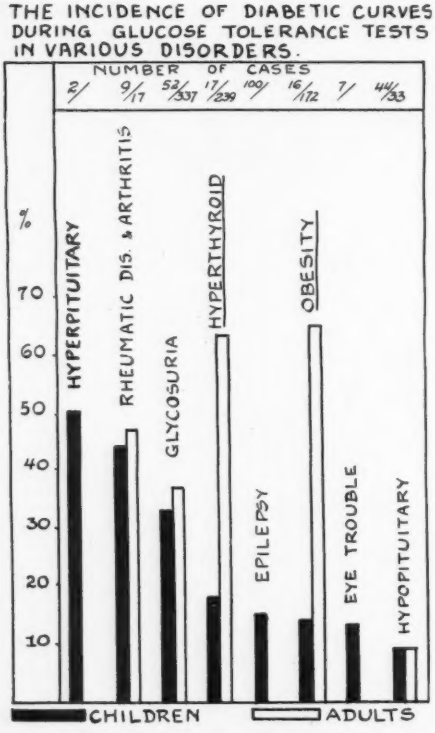
Condition	Type of Curve	Age in Years																	Total	Per Cent
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
Obesity	I							1			1	2				3		1	8	
	II										1	2					1		4	12
	III															1			1	
	IV											1							1	2
																				86
																				14
Hyperthyroid	I										1				2	3	1	1	8	
	II							2		1	1						2		6	14
	III														1				1	
	IV													1			1		2	3
																				82
																				18
Hypothyroid	I				1		1	1						1		1		1	6	
	II												1		2	1			4	10
	III																			
	IV																			
																				100
Hypopituitary and Dwarfism	I			2			1	4	4	1	2	2	1	1		7	4	1	30	
	II					1				2	2	1	1	1			2		10	40
	III											1							1	
	IV										1					2			5	4
																				91
																				9
Glycosuria	I	1				5	2			2	1		4	2	2	3	3	3	28	
	II	1												1	1	2		2	7	35
	III										1	1							2	
	IV		1	1				1	2	1	2		2			3	1	1	15	17
																				67
																				33
Hyperglycemia	I						1												1	1
	II																			
																				100
Rheumatic Disease	I							1						1			1	1	4	
	II																1		1	5
	III																	3	3	
Arthritis	IV																	1	1	4
																				56
																				44
Eye Conditions	I						1					2		1	4	1	1		10	
	II					1						3		1	2	1		1	9	19
	III														3				3	
	IV																			3
																				87
																				13
Epilepsy	I								1		1					1		1	4	
Petit Mal	II								1				1						2	6
Encephalitis	III															1			1	
Neuralgia	IV																			1
																				85
																				15
Hyperpitituary	I												1						1	1
	II																			
	III														1				1	1
	IV																			
																				50
																				50
Raynaud's Disease	I											1							1	1
	II																			
																				100
Normal	I												1						1	
	II															1			1	2
																				100
Tuberculosis	I															1	1		2	
	II												1						1	3
																				100
Skin Diseases	I													1	1			1	3	
	II														1				1	4
																				100
Pregnancy Glycosuria	I																			
	II															1			1	1
																				100
Cholelitiniasis	I														1				1	1
	II																			
																				100
Hypogonadism	I													1					1	1
	II																			
																				100
Duodenal Ulcer																		1	1	1
																				100
Total.....	*N	2	0	2	1	7	6	9	6	6	10	13	11	11	16	25	18	14	192	
	*D	0	1	1	0	0	0	1	2	1	4	3	2	1	5	7	2	5		
Per Cent.....	*N	100	0	66	100	100	100	89	75	86	71	87	85	92	76	79	90	74	Avg.	82
	*D	0	100	33	0	0	0	11	25	14	19	13	15	8	24	21	10	26	Avg.	18

*N stands for normal.

*D stands for diabetic.

other end of the chart, there is the lowest incidence of the diabetic curve; in fact, most of these show an increased tolerance. Rheumatic disease is a chronic infection, and as such, is one of the causes of juvenile diabetes.

In children with glycosuria we find that only 33 per cent have diabetes; this corresponds to the findings in adults. Hyperthyroidism in children is accompanied by diabetes but rarely, in contrast to the frequency of decreased sugar tolerance in adults with hyperthyroidism. Glucose toler-



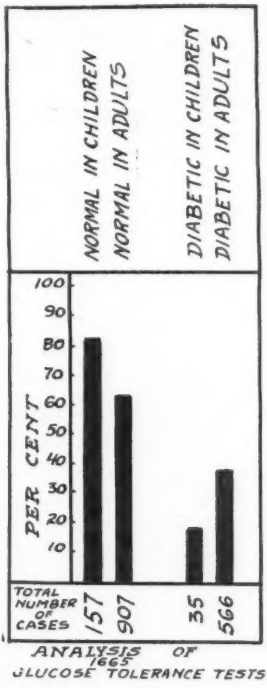
12. The incidence of diabetic glucose tolerance curves in the various disorders in children and adults.

ance tests in children with obesity show very little tendency to abnormality, which is also quite the opposite picture to that seen in adults who are obese. These show an incidence of diabetes almost seven times as great as in those of normal weight.

In a child with increased sugar tolerance, various endocrine affections such as hypothyroidism, hypogonadism, hypopituitarism have a part. While the endocrines do play a definite part in this picture, this is no reason to disregard the diabetic type of curve in these groups, for we know practically nothing about the ultimate state of these children. Given

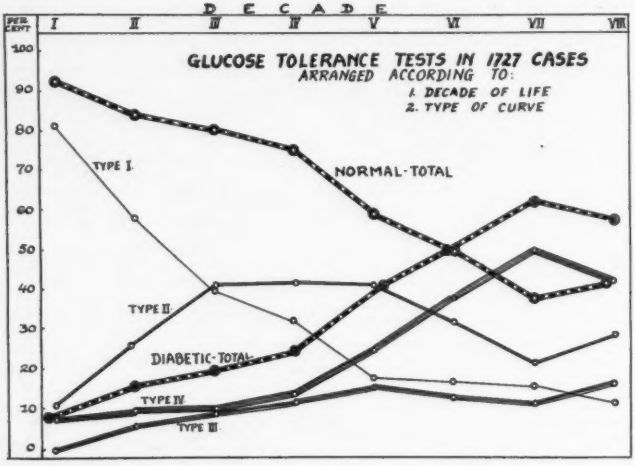
100 children with hyperpituitarism with a normal glucose tolerance curve and another 100 children with hyperpituitarism with a diabetic curve, who can say today that the incidence of diabetes in both groups in years to come will be the same? We simply do not know, and hence this presents an open problem.

In Chart 13 I have tried to correlate in a summary the results of 1665 glucose tolerance tests in children and in adults. Normal curves were obtained in 82 per cent of the children and only in 62 per cent of the



13. An analysis of 1665 glucose tolerance tests in children and adults.

adults. This suggests that diabetes must be largely a degenerative disease of advancing decades, resulting from various affections, endocrine imbalance, infections, arteriosclerosis and senescence. This fact is shown even more strikingly in Chart 14, in which 1727 glucose tolerance curves are classified according to the incidence of Types 1, 2, 3 and 4 in the various decades. The broken lines indicate the grand summary of all these types. Note the low incidence of diabetes in the first four decades, after which the rise is more abrupt. In the seventh decade the incidence is the highest and it is in this decade of life that the bodily tissues and the bodily functions are at low ebb.



14. Glucose tolerance tests, 1727 in number, arranged according to decades and according to the type of curve obtained .

SUMMARY

1. This study represents an analysis of the results of glucose tolerance tests in 192 children. The results have been compared with those obtained in a study of approximately 1500 adults. Normal curves were obtained in 82 per cent of the children as contrasted with only 62 per cent in the adult group. There was a low incidence of diabetic curves in the first four decades, after which the rise was more abrupt with the peak in the seventh decade.

2. There was no appreciable difference between the normal fasting blood sugar level in children and in young adults; the values fluctuated between 60 and 120 mg. per cent, although most fell between 71 and 110 mg. per cent.

3. Among the patients was a group of 25 children in whom diabetes developed within four to 30 days following severe infections. The data from these indicated that infections may produce temporarily decreased glucose tolerance and also may play a definite and considerable part in the production of permanent diabetes in children.

4. In 52 children with glycosuria, 33 per cent showed a diabetic type of glucose tolerance curve. Similarly, in a series of 337 adults with glycosuria, 125, or 37 per cent, showed a diabetic type of curve.

5. Only three of 17 children with hyperthyroidism showed a diabetic type of curve. This contrasts with the finding of 63.5 per cent diabetic curves in a group of 239 adults with hyperthyroidism.

6. Of 14 obese children, only two showed decreased sugar tolerance, in striking contrast to the findings in 172 obese adults, 113 or 65.6 per cent of whom showed a diabetic type of curve.

7. Some workers tend to minimize the importance of decreased sugar tolerance in children, regarding it as the result of various factors, but this finding should never be disregarded, for only by continued study of these children will the true significance of decreased sugar tolerance be revealed.

THE EFFECT OF DAILY HETERO-PITUITARY IMPLANTS INTO ADULT BUT SEXUALLY INACTIVE MALE GROUND SQUIRRELS*

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While extensive work has been done with pituitary implants in female rats and mice very little has been published on the male mammal. In contrast with the great follicular development in the ovary there has been little change reported in the histology of the testis after pituitary implants. Smith and Engle (1) showed an increased size of the male reproductive organs exclusive of the testes in young rats and mice but in adult male rats no effect of implants was seen.

Pituitary implantation into ground squirrels was begun in this laboratory in 1929 and has continued to 1932 in both sexes and to 1933 in the male. In 1931 Johnson and Wade (2) reported a change from primary spermatocyte spireme stages to actively dividing spermatocyte divisions and spermatids as a result of eight daily implants of rat pituitaries into each of two males. Microphotographs showed these changes and indicated an increase in size of the tubules. A procedure of taking a sample of one testis before the implants were made was described as necessary in the ground squirrel because of the variation in development among individuals.

Later in 1931 Moore and Price (3) confirmed Smith and Engle's (1) results, producing in four young male rats a marked effect on the size of the prostate gland and of the seminal vesicles but no consistent increase in size of the testes and tubules.

MATERIAL AND METHODS

Adult male thirteen-lined ground squirrels, *Citellus tridecemlineatus arenicola* (Howell), from Western Kansas were given daily implants of rat pituitaries. With four exceptions in the preliminary experiments, the ground squirrels were inactive sexually, i.e., the testes were small and in the abdomen and no scrotum was apparent. Abnormally fat or thin individuals were avoided. Since considerable variation in the sexual condition of the males has been observed (4), especially in the winter, it was necessary to know the exact condition of the testes before any implants were made. Each animal was therefore opened ventrally and the tunic of the right testis was punctured with a spear-pointed needle and a few tubules squeezed out and fixed as a sample before implantation. Both testes were measured in three dimensions. After three to ten days had been allowed

*Contribution No. 158 from the Department of Zoology of the Kansas Agricultural Experiment Station.

for healing, the animals were given four daily implants of rat pituitaries under the skin of the back. This series of implants was followed on the next day by a removal of the right testis for histological study and by measurement of both testes. Implantation was resumed usually about one day later, but in a few cases the animal was given three to five days in which to recover. After a total of eight, twelve or sixteen implants the animal was killed and the left testis, Cowper's glands, the prostate gland and the seminal vesicles were removed, measured, weighed and fixed for study.

In addition to the sample of the right testis serving as a control, several of the pituitary-implanted animals had as controls other males which were implanted with brain tissue and which were operated on as were the pituitary-implanted animals. This was done chiefly to check the possibility of compensatory hypertrophy of the remaining testis after the removal of the right one after four implants. The procedure given was developed during the "preliminary" experiments and was followed in detail in the later or "final" experiments.

RESULTS OF PRELIMINARY EXPERIMENTS

Since the work done in 1930-31 (Foster) and in 1931-32 (Coco) was somewhat preliminary in nature it will be considered first. The procedure differed from that just given chiefly in the number of implants given both before and after the removal of the right testis. The results of the pituitary implantations have been organized briefly in Table I. A study of Table I-A shows that 11 male ground squirrels given rat pituitary implants for three to nine days (or longer in three instances) showed in seven cases a marked increase in the size of the testes, generally most pronounced after the longer period of implantation as they were usually measured after three or four implants and again after eight or more implants. Four males given pituitary implants in the spring and summer showed no increase in size of testes and possibly a slight decrease in two cases. The latter two had about completed their regression to the sexually inactive state and it is possible that this in some way may prevent a response to pituitary implants. At any rate a male control for the one of July 11, 1931, operated on in the same way but not receiving pituitary implants had a very rapid regression from a stage slightly more active than the experimental. The last animal of June 9, 1932, had some infection resulting from its operation. This may have inhibited growth of the testis.

All but one male in Table I-A showed an increase in diameter of the tubules of the testes, eight of the eleven showing a marked increase. Six of the eleven showed a definite advance in the histological picture of spermatogenesis and all but two of them showed some advance.

In Table I-B it is seen that the implants stimulated the reproductive organs in the two males with testes originally 14 to 17 mm. long. The testes descended in both and enlarged at least in one. In the other the measure-

ment of the right testis upon removal after four implants was overlooked. It was recorded as of medium size and descended. The left testis did not descend and it was slightly smaller than the original size of the right testis, but it contained spermatozoa.

TABLE I

SUMMARY OF PRELIMINARY EXPERIMENTS OF ANTERIOR PITUITARY IMPLANTS FROM RATS INTO MALE GROUND SQUIRRELS

Date Begun	Number of Implants	Resulting Increase in			Remarks
		Size of Testes	Diameter of Tubules	Spermato-genesis	
A. Into Ground Squirrels with Small Testes (10–12 mm. long.)					
October 2, 1930.....	14 in 5 wks.	++*	++	++	Became partly scrotal.
November 28, 1930.....	7, 9	++	++	+	Scrotal after 5 implants.
January 10, 1931.....	1, 3	++	++	+?	
June 8, 1931.....	5, 12	++	++	++	Became scrotal.
July 11, 1931.....	4, 10	−?	−?	0	Regression? See control.
May 5, 1932.....	9	0	++	++	
May 9, 1932.....	4, 9	++	++	++	
May 9, 1932.....	4, 9	++	++	0	Poor fixation.
June 9, 1932.....	3	0	+	++	
June 9, 1932.....	3, 8	++	++	+?	
June 9, 1932.....	4, 9	−?	+	++	Testes slightly descended.
B. Into Ground Squirrels with Medium Sized Testes (14–17 mm. long.)					
January 30, 1932.....	4, 8	−?	++	++	Young. Spermatozoa.†
May 5, 1932.....	4, 9	+	+	+	Testes descended. Spermatozoa.
C. Into Ground Squirrels with Large Testes. (18–20 mm. long.)					
January 9, 1931.....	1, 3	+	+	+	Spermatozoa freer in lumina.
January 27, 1931.....	3, 5	−?	+	+	More spermatozoa after 3 implants; fewer after 5.
D. Control Animals, Operated on but not Implanted.					
December 6, 1930.....	8, 13	0	0	0	
July 11, 1931.....	5, 12	−	− −	− −	Rapidly regressing.
July 11, 1931.....	5	++	0	0	Testes increased 2×1.5×0 mm.
June 9, 1932.....	11	− −	0	−	

*Explanation: ++ Indicates a decided increase. + A small increase. 0 No change. − a small decrease. +? or −? Increase or decrease not definite, slight, about the same as no change.

† Right testis descended; left contained spermatozoa.

From Table I-C it is suggested that pituitary implants for three to five days have very little effect on testes which are already large, but do cause a slight enlargement of the tubules and perhaps a slight hastening of the freeing of the spermatozoa into the lumen of the tubule and perhaps the elimination of them from the lumen.

Four controls are shown in Table I-D. These received the same operative treatment as the experimental animals except that they were not implanted with pituitary glands. The control in December showed no change. One in July, 1931, showed a marked retrogression and another showed no change except that the testis had increased 2x1.5x0 mm. in dimensions. The fourth animal (June, 1932) showed a decrease in testis size and in

spermatogenesis. It appears probable that the testes were regressing at this time of the year and this probably helps account for the slight response in a few of the experimentals at this time of the year.

RESULTS OF FINAL EXPERIMENTS

Effects on Testes

In 1932-33 the implant work on the male ground squirrel was carried on more intensively (Gann). Twelve adult males with small testes were given eight to sixteen pituitary implants as described under "methods" and each served as its own control as to size and development of the testes, the right testis being removed after four implants and the left testis and the accessory organs after eight implants (four animals) or 12 implants (six animals) or 16 implants (two animals). Four other males served as special controls to determine whether the operation or the time of year might cause a development of the reproductive system. They were operated on and received implants of brain tissue. It should be noted that the experiments were conducted at a time of year when natural sexual development would not be expected to occur (4), viz., in October, November, and early December and in June.

The results of the work on the 12 pituitary implanted animals are summarized in Table II, which also includes statistical treatment to show whether the changes produced by the implants are significant. For the sake of completeness the four animals given brain implants are included, but it is realized that too much dependence should not be placed on the comparisons with these four animals because of their small number and because they are not as reliable controls as were the 12 experimental animals themselves before they received any implants.

Size. After four daily implants all twelve animals showed an increase in size of the right testis of more than 1 mm. in each of two dimensions, except in one animal in which it was 1x0.5 mm. Eleven showed an increase of the left testis more than the equivalent of 1 mm. in each of two dimensions except that in one testis this increase was in only one dimension. One left testis showed no change in size.

As a result of the four pituitary implants the right testis increased from 10.2x5.0x4.5 mm. to 13.5x6.5x5.8 mm.; and the left from 10.1x5.5x4.7 mm. to 13.5x6.6x5.9 mm. In Table II (lines 1 and 2) these dimensions are multiplied to give the approximate relative volumes. It is to be seen that the average volume of the 24 testes changed from 265 cu. mm. to 550 cu. mm., a difference which is seen to be statistically very significant.

In two of the four animals receiving brain implants both testes retained the original size. In one the right testis decreased 0.5 mm. in two dimensions and the left increased 0.5 mm. in one dimension. In the fourth the right testis increased the equivalent of 1 mm. in each of two dimensions but the left did not change. The four brain implants produced no significant result (Table II, lines 3 vs. 4). Although the brain-implanted animals

TABLE II

Effect of Pituitary Implants on Male Ground Squirrels

Line No.	No. of Glands	Glands or Tubules	Implants No. Kind	Range of Vol. Diam. or Weight	Mean of Vol. Diam. or Weight	S. E. of Mean	S. D. of Distrib.	Lines Compared	Diff. Means	S. E. Diff.	Diff. / S. E. Diff.
1	24	Testes	4 Ant. Pit.	198–1088	550 cu. mm.	52.7	258.3				
2	24	Testes	0 Ant. Pit.	38– 546	265 cu. mm.	27.3	134.2	1 vs. 2	285.0	59.4	4.80*
3	8	Testes	4 Brain	44– 546	334 cu. mm.	58.9	166.8	1 vs. 3	216.7	79.1	2.74
4	8	Testes	0 Brain	63– 504	313 cu. mm.	52.3	148.1	3 vs. 4	20.4	72.2	0.26
5	12	L. Testes	8–16 A. P.	330–1454	922 cu. mm.	98.3	340.6				
6	12	L. Testes	4 Ant. Pit.	198–1088	570 cu. mm.	22.1	265.0	5 vs. 6	352.9	100.8	3.50
7	4	L. Testes	8–16 Brain	108– 840	459 cu. mm.	120.7	261.5	5 vs. 7	462.9	155.7	2.97
8	4	L. Testes	4 Brain	86– 546	347 cu. mm.	83.0	166.1	7 vs. 8	112.5	146.5	0.77
9	12	Tubules	4 Ant. Pit.	87– 155μ	126μ	6.7	23.3				
10	12	Tubules	0 Ant. Pit.	57– 121μ	101μ	5.4	18.7	9 vs. 10	24.9	8.6	2.89
11	4	Tubules	4 Brain	65– 138μ	107μ	13.3	26.7	9 vs. 11	18.8	14.9	1.27
12	12	Tubules	8–16 A. P.	111– 166μ	135μ	4.3	14.8	9 vs. 12	9.6	8.0	1.20
13	12	Prostates	8–16 A. P.	20– 55 mg.	32 mg.	2.8	9.6				
14	4	Prostates	8–16 Brain	9– 23 mg.	14 mg.	28.0	5.6	13 vs. 14	18.8	3.9	4.78
15	12	Cowper's	8–16 A. P.	20– 140 mg.	44 mg.	8.1	28.0				
16	4	Cowper's	8–16 Brain	5– 15 mg.	10 mg.	1.9	3.8	15 vs. 16	33.8	8.3	4.03
17	12	Sem. Ves.	8–16 A. P.	50– 270 mg.	100 mg.	16.5	57.2				
18	4	Sem. Ves.	8–16 Brain	12– 25 mg.	18 mg.	2.5	4.9	17 vs. 18	81.8	16.7	4.90

*A value of 2.7 or over is considered significant when standard error (S. E.) and standard deviation (S. D.) are used.

had larger testes than the pituitary-implanted animals before either group was implanted, it is to be seen that those receiving the four pituitary implants had testes significantly larger than those which had received four implants of brain tissue (lines 1 vs. 3).

All four of the animals killed after eight pituitary implants showed an increase in the size of the left testis over the size after four implants, and in three of them the increase was more than 1 mm. in each of two dimensions. The average increases of the dimensions were 1.2x1.3x0.5 mm. The single control showed no increase in size from the four to eight brain implants.

All six of the animals killed after 12 pituitary implants showed increases in the size of the left testis, over that found in these testes after four implants, averaging 2.7x1.3x1.3 mm. in the three dimensions. One brain implanted control showed a slight decrease and one a slight increase in size but not more than the equivalent of 1 mm. in each of two dimensions.

Two animals were given 16 pituitary implants each. One showed increases of 3x3x2 mm. and the other 3.5x3x2.5 mm. in dimensions of the left testis over the size after four implants. Since the control animal given 16 brain implants showed increases of 2x2.5x2 mm. in the three dimensions, it would appear possible that some of the increase was produced by a compensatory hypertrophy caused by the removal of the right testis after the four implants, for 13 days had elapsed from the time of removal of the right testis to the time of autopsy in the case of the experimental animals and 14 days in the case of the control.

If compensatory hypertrophy does not occur in males, the increase in the control and part of that in the two experimentals must be attributed to an unusually early seasonal sexual development such as sometimes occurs (4). A definite increase could be manifested in two weeks.

In Table II all the animals given more than four pituitary implants are treated together as each group would be too small by itself to be treated statistically. It is seen that the left testes made significant gains in size beyond the size after four implants (5 vs. 6). In spite of the fact that one of the controls given 16 brain implants showed the increase already mentioned, and the brain group average was thereby raised, it is seen that the 12 animals given 8-16 pituitary implants had significant increases in size as compared to the four controls given 8-16 brain implants (5 vs. 7) but the latter showed no significant increase in testis size from that after four brain implants (7 vs. 8).

Diameter of Tubules. All of the pituitary implanted animals showed an increase in diameter of the seminiferous tubules ranging from 14μ to 46μ, and averaging 27μ, after four implants. This increase is significant (Table II, 9 vs. 10) but probably more so than indicated by statistical treatment since the increase was shown by all of the experimental animals and no change was shown by the four controls after the different numbers of brain tissue implants.

After eight, twelve and sixteen implants there was a great further increase (over 12μ) in tubule diameter in only five out of twelve cases over the size after the four implants. In four others there probably had been an increase but the tubules had been shrunken by the technique. Three others showed no further increase. It appears that a continuation of implantation beyond four usually, but not always, promotes further growth of the tubules, but the evidence is not conclusive (Table II, 9 vs. 12).

The tubule diameter for each animal was determined by averaging approximately 30 measurements of representative tubules, made ten each by Gann, Burton Baker and the senior author. The averages of the measurements of the three observers varied only slightly.

Histology of Tubules. A careful histological study was made of the sample of the right testis removed as a control piece before the animal was given either pituitary implants or brain implants. In practically every

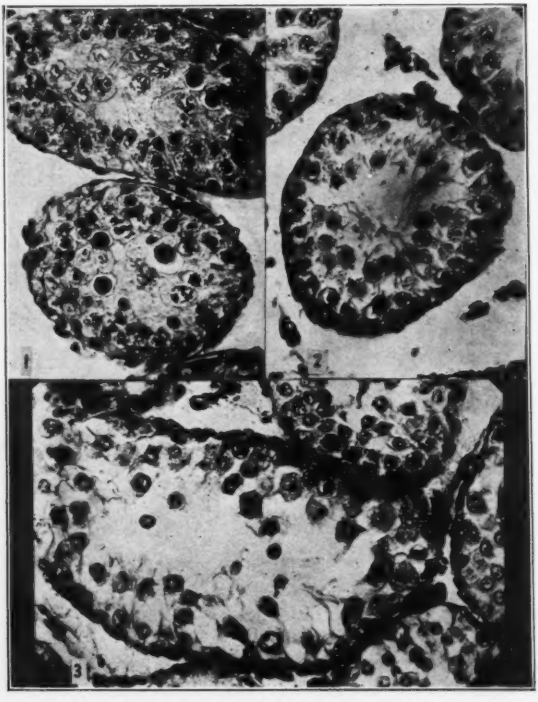

Figure 1. Seminiferous tubules of the right testis of ground squirrel No. 188 before pituitary implantation, showing spermatogonia peripherally and primary spermatocyte spiremes centrally. No lumen is present. X213. (*Note*: We are indebted to Mr. Charles G. Dobrovolny for taking the photomicrographs.)

Figure 2. Tubules of the right testis of ground squirrel No. 188 after four daily pituitary implants, showing lumen formation and, in the tubule in the center, late telophase stages of the first maturation division, and increased tubule diameter. X213.

Figure 3. Tubules of the left testis of ground squirrel No. 188 after 16 pituitary implants, showing lumina, first maturation division stages, secondary spermatocytes and increased tubule diameter. X213.

case there were present only spermatogonia and primary spermatocyte spiremes (Fig. 1). The same testis removed after four daily pituitary implants showed in every case primary spermatocyte division stages and secondary spermatocytes (Fig. 2). Division of the secondary spermatocytes and the presence of spermatids were each observed once. A lumen appeared in a varying number of tubules in about three-fourths of the animals. It was usually small. Brain implants for four days in the control animals caused no change in the histology of the testes except the production of small lumina in one-half of the tubules of one animal.

After eight, twelve, or sixteen rat pituitaries had been implanted (daily, except for one or two days rest at the time of removal of the right testis after the first four implants) the left testis showed a definite histological advance over the four implant condition of the right testis in only four cases, but in two of these spermatozoa were actually formed and the testes were enlarged to about breeding-season size and were in the scrotum, which was more or less pigmented. In three other cases there was a very slight advance histologically. The most marked change in the tubules resulting from the longer implantation period was the enlargement of the lumina and what appeared to be an elimination of the contents of the tubule (Fig. 3). However, the stages in maturation were not reduced but the number of cells present were.

The testes of the two males which were scrotal in position and contained spermatozoa had the lumina of many tubules filled with secretion and spermatozoa. It seems quite possible that the large clear lumina of the testes that remained in the abdomen were due to the high body temperature as has been shown for some other mammals (5).

No change in interstitial tissue was observed as a result of pituitary implantation.

Effect on the Accessory Glands

Implantation of anterior pituitary glands into sexually inactive male ground squirrels caused a very marked hypertrophy of the prostate glands, Cowper's glands and the seminal vesicles. The average weights of the four control prostate glands was 14 mg. and of the 12 experimentals was 32 mg. The control Cowper's glands averaged 10 mg.; the "experimentals" averaged 44 mg. The control seminal vesicles averaged 18 mg., and the "experimentals" averaged 100 mg. All of these differences were found on statistical test to be significant (Table II). It was not practicable to remove these glands in the living animal. The glands were weighed and fixed for histological study at the time of autopsy, i.e., after eight implants in four animals, after 12 implants in six, and after 16 implants in two. A study of the weights of the glands revealed little, if any, constant increase in the glands of the animals receiving 12 and 16 implants over that of the animals receiving eight implants.

Histological examination of the prostate glands of the sexually inactive control animals showed lumina of ducts in only a few (Fig. 4). Usually the sites of the ducts were marked only by compact groups of darkly stained secretory cells within somewhat concentric rings of lightly stained non-secretory cells. Pituitary implanted animals showed large lumina, lined by a low cuboidal epithelium (Fig. 5).

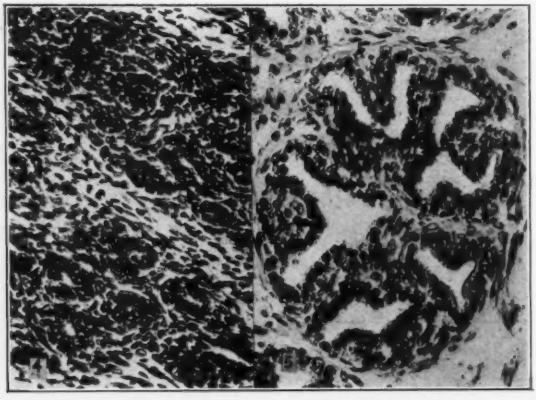

Figure 4. Prostate gland from control animal No. 1165, which had received eight implants of brain tissue. X213.

Figure 5. Prostate gland from animal No. 1713, which had received 12 pituitary implants. X213.

In the sexually inactive controls, the bulbo-urethral glands were small. The secretory ducts were lined with a low cuboidal epithelium of which the cells were very little larger than the nuclei (Fig. 6). The average height of

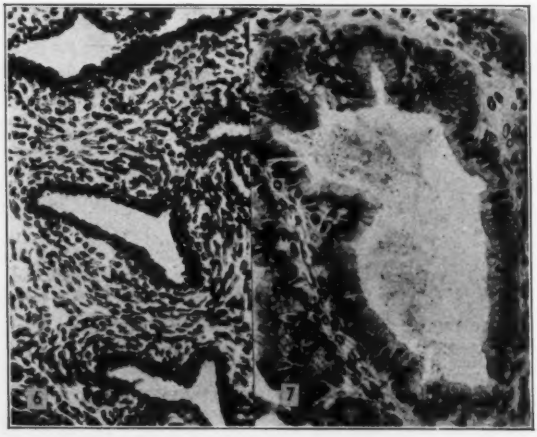

Figure 6. Cowper's gland from animal No. 1165, which had received eight implants of brain tissue. X213.

Figure 7. Cowper's gland from No. 1713, after 12 pituitary implants. X213.

the secretory epithlium was about 7 micra. In contrast, Cowper's glands of the experimental animals had enlarged enormously, the acini spaces were distended with secretion, and the secretory epithelium had become greatly proliferated and averaged from 16 to 18 micra in thickness. The cells were usually tall columnar and the nuclei were located basally (Fig. 7).

The seminal vesicles of the control animals were characterized by rather small ducts lined with a low cuboidal epithelium about 7 to 9 micra in thickness (Fig. 8). After implantation with pituitary glands the ducts had enlarged and were usually filled with secretion. The epithelial layer consisted of columnar cells and averaged about 20 micra in width (Fig. 9). The nuclei were elongated and were basal in position.

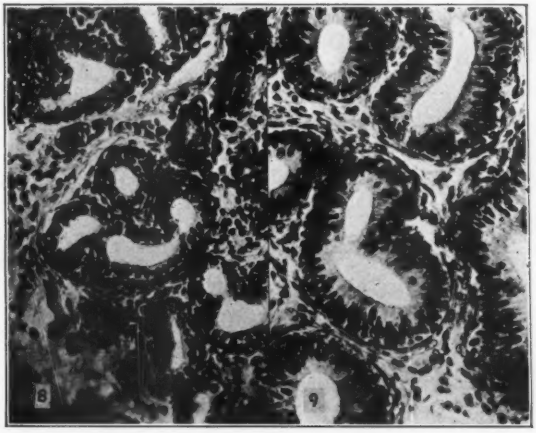

Figure 8. Seminal vesicle from No. 1165, after eight implants of brain tissue. X213.
Figure 9. Seminal vesicle from No. 1713, after 12 pituitary implants. X213.

Effect on Weights of Animals

A study of the weights of the animals in these experiments reveals the fact that fat animals (140 gm. or more) lost considerable weight when given pituitary implants in contrast with the controls which were under the same conditions. Thinner animals weighing around 100 gm. were able to hold their weight or make slight gains. It appears that the pituitary implants raised the metabolism of the animals for they were much more active and resisted handling more vigorously than did the controls. Similar losses in weight and increases in activity have been observed in other individuals given pituitary implants in hibernation experiments.

SUMMARY

Experiments are reported that show conclusively for the first time an effect of pituitary implants upon the testes of adult male mammals. Four

daily implants of rat anterior pituitary glands into 12 sexually inactive but adult male ground squirrels produced a marked enlargement of the testes (28 per cent average increase of three dimensions, or over 100 per cent increase in volume) and seminiferous tubules (25 per cent increase in diameters), and caused a change in the cells of the latter from primary spermatocyte spireme stages to first maturation divisions, secondary spermatocytes, second maturation divisions (one animal) and spermatids (one animal). Lumina usually appeared in the tubules. These comparisons are between pieces of the right testis taken before and after the four implantations.

Continuation of the implantations up to a total of eight, twelve or sixteen in the above animals produced further advances in size of testes and tubules (including lumen formation) and in spermatogenesis (in two cases to formation of spermatozoa) but these additional changes were not so great nor so constant as those already produced by the first four implants. After eight to sixteen pituitary implants the accessory reproductive organs were greatly increased in weight over those of controls given the same number of implants of brain tissue. These increases were: prostate, over two times; Cowper's gland, over four times; seminal vesicle, over five times. The glands also showed a great advance in cellular development indicating a change from rest to active functioning.

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BLOOD CHEMISTRY OF ADRENAL INSUFFICIENCY IN CATS†

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The purpose of the experiments described in this paper was to find some criterion for the level of activity of the adrenal cortex similar to the tests now in use for other endocrine glands, such as blood sugar level for the islands of Langerhans, basal metabolism for the thyroid, or calcium and phosphorous values for the parathyroid.

Changes in the blood chemistry of animals suffering from adrenal insufficiency have been reported by many investigators using cats (1) (2) (3) (4) (5), dogs (6) (7) (8) (9) (10) (11), and rats (12) (13). The variety of constituents studied and the variable results obtained led us to check a number of the more striking results. A composite picture proved to have a good critical value in the determination of adrenal insufficiency, but an increase in serum potassium and a loss of Na and Cl seem to us to have the greatest specific value.

Adult male cats were used for most of the experiments. They were kept in separate cages and fed a diet of canned salmon and milk. This diet was supplemented with fresh meat, cod liver oil and dried yeast.

The blood samples were always obtained directly from the heart. There is difficulty in obtaining samples elsewhere from animals with adrenal insufficiency, because of the decreased peripheral circulation. Even large limb vessels have a sluggish flow. We believe this is due to a diminished plasma volume occurring with the general dehydration (Zwemer, 14) of the animal, since there is an increase in the hematocrit reading and the red cell count. Wyman and Tum Suden (15) find there is a decrease in blood volume in adrenalectomized rats and Donath (16), Lucas (6) and Hartman, et al. (5), find an increase in total blood solids in adrenal insufficiency.

Chemical Technique. The blood for CO_2 was collected in an oiled syringe. A small sample for NPN, sugar and chlorides was put in an oxalated tube. The rest was allowed to clot under oil in an acid-washed pyrex centrifuge tube and immediately centrifuged. The serum was then transferred to a paraffined tube. At first, paraffined tubes were also used for collecting the blood, but since a check showed no difference in analysis of samples collected in paraffined tubes or in specially cleaned pyrex tubes, the latter were used in subsequent collections. These tubes were heated to about 100° C. in sulphuric acid-dichromate cleansing mixture, then thoroughly rinsed with tap water and finally with distilled water.

†Preliminary reports on parts of this work have been made:

Zwemer, R. L. and R. C. Sullivan: A blood chemical test for adrenal cortex extracts. Proc. Soc. Exper. Biol. & Med. **28**: 723. 1931.

Sullivan, R. C., A. B. Maclean and R. L. Zwemer: A comparison of the chemical composition of the blood in acute intestinal intoxication and experimental adrenal insufficiency in animals. Am. J. Dis. Child. **43**: 1279. 1932.

The following methods were used in the analyses:

NPN—determined by the micro-method of Folin using 2 cc. of tungstic acid filtrate.

Sugar—Folin-Wu (17).

Chlorides—In part of the work, in which the primary interest was the acid base balance of the blood, the chlorides were analyzed by the method of Van Slyke (18). In a great part of the work, however, the chlorides were estimated upon the Folin-Wu filtrate by the method of Whitehorn (19). This was found to check satisfactorily with the method of Van Slyke and offered the advantage of requiring a smaller sample of blood or plasma.

CO_2 capacity—Van Slyke and Neill (20).

P—Benedict and Theis (21).

Ca—Clark-Collip modification of Kramer-Tisdall (22).

Mg—Briggs (23). An aliquot of the supernatent fluid after precipitation of calcium oxalate was used instead of all the supernatent fluid and washings.

Na—Rourke's modification of Kramer-Tisdall (24).

K—Kerr's modification of Kramer-Tisdall (25).

The figures on the normal blood chemistry of the cat were obtained over a period of two years (Table 1). Repeated control samplings from

TABLE 1

BLOOD CHEMISTRY OF NORMAL CATS

No. of Cats	No. of Detrm.	Constituent		Per 100 c.c.	Maximum	Minimum	Mean	Average	Range in Which Greatest No. Detm. Fall
45	75	Blood	NPN	MG	72.3	34.5	53.4	51.6	40.0– 65.0
56	95	Blood	Sugar	MG	137.0	70.0	104.0	106.0	80.0–120.0
45	75	Blood	NaCl	MG	696.0	553.0	625.0	644.0	600.0–675.0
20	25	Plasma	NaCl	MG	768.0	657.0	712.0	703.0	670.0–740.0
62	95	Plasma	CO_2 Cap.	Vol.	47.4	27.0	37.2	36.1	30.0– 44.0
20	30	Plasma	P	MG	8.0	4.3	6.1	5.9	5.0– 7.5
20	41	Serum	Ca	MG	12.8	9.7	11.3	10.8	10.0– 12.0
18	23	Serum	Mg	MG	2.57	1.32	1.94	1.83	1.4– 2.2
18	27	Serum	Na	MG	401.0	335.0	368.0	373.0	360.0–385.0
21	33	Serum	K	MG	54.5	27.2	40.8	38.3	30.0– 45.0
		*Serum	Total Base	mM				175.4	167.3–182.8

*Sum of individual bases.

untreated animals showed only slight hourly and daily deviations. These normal figures form the basis on which we evaluated our results in this and subsequent papers; this accounts for the large number of controls used. Since some of the animals, on first being tied down, became excited, the determinations on samples obtained during excitement are not included in the normal figures.

Our average figures for blood NPN and sugar are in accord with those given in the literature. However, the values we have obtained for acids and bases of the blood in normal cats are somewhat at variance with those previously reported (Table 2). This lack of agreement, which will be taken up in greater detail in the discussion, may be accounted for by variations in the animals used as controls and in the handling of the blood sample.

The immediate and delayed effect of adrenal ablation on the concentrations of the various blood constituents was our next consideration. Removal of both adrenals results in death for the cat in six to 14 days in

TABLE 2

BLOOD CHEMISTRY OF NORMAL CATS AS REPORTED IN THE LITERATURE
(Quantity per 100 cc. Averages computed from figures given)

	Marshall, E. K. and Davis, D. L. 1916	Bauman, E. J. and Kurland, S. 1926–27A	Swingle, W. W. 1927C	Swingle, W. W. and Eisenman, A. 1927D	Hartman, F. A. et al. 1927F
Blood NPN (mg.)			44.1	47.0	
No. of determinations			(11)	(10)	
Blood Urea (mg.)	30–45		21.6		25.4
No. of determinations	(28)		(7)		(4)
Blood Sugar (mg.)			111		
No. of determinations			(12)		
Blood NaCl (mg.)		605			
No. of determinations		(11)			
Plasma NaCl (mg.)		685		651	
No. of determinations		(11)		(11)	
Plasma CO_2cap. (vol.)				43.8E	
No. of determinations				(11)	
Plasma P (mg.)		5.8	7.2	6.5	8.1
No. of determinations		(10)	(14)	(11)	(4)
Serum Ca (mg.)		10.2	9.9		
No. of determinations		(10)	(9)		
Serum Mg (mg.)		2.4			
No. of determinations		(11)			
Serum Na (mg.)		375	345		
No. of determinations		(10)	(7)		
Serum K (mg.)		19.0			
No. of determinations		(10)			
Serum Total Base (mM)		171.5B		162.8	
No. of determinations		(10)		(11)	

A. Determination of bases done on plasma (blood treated with lithium citrate).
B. Calculated by addition of individual bases.
C. Animals had had one adrenal removed.
D. Eight of eleven animals were bled under urethane anesthesia.
E. Calculated from bicarbonate by formula of Peters, et al., J.B.C., 67:141, 1926.
F. Complete data on normal figures not given.

uncomplicated cases (Zwemer and MacMahon, 26). Blood sampling tends to shorten this survival period somewhat, but with care we were able to get a number of samples from the same animal at varying periods after the operation and during different degrees of adrenal insufficiency as shown by the observable symptoms. The sample protocol of cat L41 shows how the blood chemical changes are concomitant with the clinical observations.

CAT L 41

STRAIGHT ADRENAL INSUFFIENCY

Date	CO_2	Sugar	Bl. Cl.	NPN	P	Na	K	Ca	Mg	Weight
3/ 5/31	35.8	98	658	43.8	5.4	373	38.3	10.8	1.83	2.438
3/19/31	32.0	91	613	41.6	7.3	360	45.5	11.0	2.09	2.381
3/27/31	24.8	89	613	66.7	7.6	346	44.5	11.0	2.91	2.182
3/29/31	21.2	91	592	88.4	9.2	286	115.0	10.8	3.08	2.154

3/21/31 Removed Right Adrenal.
3/23/31 Removed Left Adrenal.

On 3/27 the cat had a dry skin fold, refused food, had a red gum line, the hair was up, rough and falling out, the eyes were sunken.

On 3/29 the cat was flat on its side, looked done for, did not have to tie down to bleed.

We then gave three injections of 3 c.c. each (Ad XI HCl) cortical extract.

The cat showed immediate improvement after the second dose, the skin was more elastic, but its legs were still weak when it walked.

After the third injection the cat drank some milk and seemed much stronger.

Striking changes in blood chemistry occur within 48 hours of death and were found in nearly all the constituents studied, but the indicated direction of the change may be observed for 4 or 5 days before death. The earliest signs of adrenal insufficiency seem to be the loss of Na and Cl and the rise in K and NPN. The decrease in blood sugar and rise in Mg are indicative of further insufficiency. We fail to find important changes in Ca at any time.

In Table 3 the figures from samples taken from 15 adrenalectomized cats at various times are given. In contrast to our normal averages (Column A) are the averages from early adrenal insufficiency (Column B),

TABLE 3

BLOOD CHEMISTRY IN COMPLETELY ADRENALECTOMIZED CATS

Constituent	Per 100 c.c. of Blood	Average in Normal Cats*	Early Insufficiency		Within 48 Hours of Death		Extreme Individual Figure Obtained
			No. of Det.	Average	No. of Det.	Average	
Blood NPN	mg.	51.6	17	73.9	8	111.0	137.0
Blood Sugar*	mg.	106.0	32	86.8	20	61.7	44.0
Blood NaCl	mg.	644.0	16	587.4	10	550.0	470.0
Plasma NaCl	mg.	703.0	9	665.7	3	623.0	614.0
Plasma CO_2Cap	vols.	36.1	27	31.4	11	26.6	18.4
Plasma P	mg.	5.9	22	6.9	11	8.8	18.9
Serum Ca	mg.	10.8	14	11.0	5	10.8	11.5
Serum Mg	mg.	1.83	12	1.87	5	3.17	4.68
Serum Na	mg.	373.0	14	330.7	6	315.0	286.0
Serum K	mg.	38.3	15	58.3	7	93.9	129.9
Serum Total Base	mM.	175.3	**	163.1		165.4	162.4

*See Table 1.

**Calculated by addition of individual bases.

and those from within 48 hours of death (Column C). The individual extreme figures that may be reached are also given (Column D) and show that conditions grow progressively worse with the approach of death. The findings of other investigators in relation to adrenal insufficiency in the cat are given briefly in Table 4.

Having determined the course of events usually following adrenal removal, our next concern was whether these could be arrested or perhaps even reversed by treatment. Extracts of beef adrenal cortex (Zwemer, Agate and Schroeder, 27, and Zwemer, Agate and Sullivan, unpublished, 28) given to 17 adrenalectomized cats brought about an improvement in their blood chemistry and in their directly observable symptoms such as appetite, weight and activity. These animals were bled at various times for chemical determinations, but the wide variation in the conditions of the individual tests makes tabulation of the results difficult; however, Table 5 and the subsequent protocols give the essential findings. We were chiefly interested in the effects of the extracts on blood chemistry, and since we felt sure we were working against long survival by removal of blood and by frequent handling of the animal no very prolonged treatment was attempted.

TABLE 4

BLOOD CHEMISTRY OF ADRENALECTOMIZED CATS AS REPORTED IN THE LITERATURE
(Average determinations given in values per 100 cc. of blood)

	Marshall, E. K. and Davis, D. L.	Bauman, E. J. and Kurland, S. A	Swingle, W. W.	Swingle, W. W. and Eisenman, A.	Hartman, F. A. et al D
Blood NPN (mg.).....			89.6	83.0	
No. of determinations..			(11)	(7)	
Blood Urea (mg.).....	Twice normal		71.6		Twice normal
No. of determinations..	(16)				(25)
Blood Sugar (mg.).....			53		50% of normal
No. of determinations..			(12)		
Blood NaCl (mg.).....		556			
No. of determinations..		(9)			
Plasma NaCl (mg.).....		623		661	
No. of determinations..		(8)		(8)	
Plasma CO_2cap. (vol.)....				26.4C	
No. of determinations..				(6)	
Plasma P (mg.).....		6.3	11.5	9.1	18.9
No. of determinations..		(9)	(14)	(8)	
Serum Ca (mg.).....		11.5	10.6		10.4
No. of determinations..		(12)	(9)		(13)
Serum Mg (mg.).....		3.2			
No. of determinations..		(12)			
Serum Na (mg.).....		320	325		
No. of determinations..		(12)	(7)		
Serum K (mg.).....		28.0			
No. of determinations..		(11)			
Serum Total Base (mM)..		150.5B		155.8	
No. of determinations..				(7)	

A. Determination of bases done on plasma (blood treated with lithium citrate).
B. Calculated by addition of individual bases.
C. Calculated from bicarbonate by formula of Peters, et al., J.B.C., 67:141, 1926.
D. Data not given in detail.

TABLE 5

EFFECT OF ADRENAL CORTEX EXTRACT ON THE BLOOD CHEMISTRY OF ADRENALECTOMIZED CATS

Constituent	Per 100 c.c. of Blood	Average Before Extract	Average Soon After Injection	Result of Injection
Blood NPN	mg.	79.3	72.5	Fall
Blood Sugar	mg.	94.7	170.0	Rise
Blood NaCl	mg.	571.0	584.3	Rise
Plasma CO_2(Cap.)	vols.	31.1	36.9	Rise
Plasma P	mg.	6.7	6.1	Fall
Serum Na	mg.	324.0	352.0	Rise
Serum K	mg.	73.8	31.9	Fall

With all our active extracts, both the observed symptoms and blood chemical changes were favorably affected. The results following injections of extract at increasing degrees of adrenal insufficiency, i.e., 2, 4 and 7 days after complete adrenal removal, are shown in the protocols of cats L21, L12, and L50.

The effect of an injection of adrenal cortex extract on the blood chemistry of adrenalectomized cats at various periods after adrenalectomy.

CAT L 21 (Two Days)

		Weight
11/19/30	Both adrenals were removed through a mid-abdominal incision. The animal showed no shock, and had a rapid recovery from the operation	5.528
11/21/30	A blood sample taken two days after double adrenalectomy showed a normal picture.	5.500

	CO_2	Bl. Sugar	Bl. Cl.	NPN	K
10:55 A.M.	36.0	112	605	48.0	27.2

10:40 A.M. An injection of 1 c.c. of adrenal cortex extract was given, and blood samples taken at intervals.

12:10 P.M.	35.6	167	631	37.5	22.6
5:10 P.M.	44.4	100	642	57.6	35.8

11/22/30 Another c.c. of extract was given. Its weight on 11/23/30 was 5.508

The above changes in the blood are similar to those occurring in normal cats after an injection of extract. These will be discussed in a future paper. This cat was subsequently used for other tests and finally died at 2:50 P.M. on 11/29/30.

CAT L 12 (Four Days)

		Wt. in Kg.
10/17/30	Removed left adrenal	4.054
11/ 3/30	Removed right adrenal	4.195
11/ 7/30	Four days after complete adrenalectomy the cat refused solid food and an analysis of its blood gave the following results	4.040

	CO_2	Sugar	NPN	P
1:30 P.M.	35.4	98	76.6	6.6

1:40 P.M. Injected 2 c.c. extract.

11/ 9/30

11:15 A.M. Injected 2 c.c. extract.

11:50 A.M.	A blood sample was taken and showed	4.026

CO_2	Sugar	NPN	P
39.0	101	57.3	6.7

11/10/30

10:55 A.M.	This animal was unfortunately killed while obtaining another blood sample from the heart	3.976

CAT L 50 (Seven Days)

		Wt. in Kg.
5/22/31	Removed both adrenals by mid-abdominal incision	3.883
5/23—5/25	Good recovery, cat ate well.	
5/26/31	Did not eat all its salmon. Hair beginning to fall out. Some diarrhea present	3.799
5/27/31	Cat refused solid food and took only 75 c.c. of milk.	
5/28/31	Still sits up, so did not inject.	
5/29/31		
10:20 A.M.	Refused all food and drink.	
11:40 A.M.	Took 10 c.c. of blood for analysis	3.629

CO_2	Bl. Sugar	Bl. Cl.	NPN	Na
30.6	71	531	110.2	327

11:45 A.M. Injected 3 c.c. adrenal cortex extract.

2:40 P.M. Took blood again, three hours after injection.

CO_2	Bl. Sugar	Bl. Cl.	NPN	Na
35.0	102	567	104.2	352

The blood chemical reaction to extract occurs even after severe insufficiency.

Severe insufficiency (anorexia for 24 hours, extreme weakness, and definite blood chemical changes) was allowed to develop in some cases before the animals were given treatment. In others, the treatment was begun soon after the operation, and the blood showed no such change as occurred after adrenalectomy without treatment. When the treatment was stopped, the animals died within a few days with typical symptoms and blood changes. Another group of animals were used to test the effect of the extract on observable symptoms, after insufficiency had been established by

chemical means (Cat L47). In still another group, only observable symptoms were used as criteria. These are: weight loss, a dry skin fold, red

CAT L 47

		Weight
2/28/31	10 c.c. of blood was taken for normal chemistry with the following results.....	4.479
	CO_2 35.0 Sugar 100 Blood Cl. 643 NPN 51.8 P 5.8 Na 361 K 40.0 Ca 10.8 Mg 1.48	
3/12/31	The first adrenal was removed.....	4.421
5/23/31	The second adrenal was removed.....	4.536
3/26/31	10 c.c. of blood was taken and the analysis showed early insufficiency, although the animal was apparently in good condition.	
	CO 27.2 Sugar 93 Blood Cl. 618 NPN 120 P 8.2 Na 355 K 102.5 Ca 11.8 Mg 2.35	4.521
3/27/31	The animal refused food, so treatment was begun.	
3/31/31	Cat eats fairly well and seems quite active.....	4.593
4/ 4/31	Extract was discontinued and the animal died 48 hours later, 4/6/31.....	4.280

gum line, falling hair, asthenia, anorexia, etc. For example, cat L66 was observed until symptoms of insufficiency appeared. Injection of extract was followed by an increase in weight, a return of appetite and playful activity. Its hair stopped falling out and the red gum line was no longer visible.

CAT L 66

To show that the effects of the extract are not transient phenomena, the following sample protocol is presented:

		Weight
L66	Right adrenal removed 4/7/31.....	3.373
	Left adrenal removed 4/24/31.....	3.345
	Symptoms appeared four days later with refusal of food and loss of weight, 4/28/31	3.288
	Daily injections of extract Ad XII were given and there was an immediate improvement in appetite and a gain in weight, 5/3/31.....	3.573
	This was maintained for three days more, then extract was discontinued, 5/6/31..	3.573
	Four days later the weight had dropped and the animal died, 5/10/31.....	3.345

We feel that blood chemistry is a good indicator of insufficiency, but no one constituent can be completely depended on as yet. A composite picture is safest. We have checked on three clinical cases of Addison's disease with the cooperation of the Department of Medicine and found the chemical picture of low CO_2 capacity, high NPN or urea, blood sugar in the lower limits of normal, and low chlorides and bases. These were returned to more normal conditions and the appetite and feeling of well-being of the patients were restored by injections of our extract. They will be reported in detail when we have sufficient additional cases.

DISCUSSION

Many of the blood constituents of the normal cat have been determined on a large number of animals (Table 1). Our variance with the findings of others (Table 2) may be due to following differences in technique.

Bauman and Kurland made their analysis for chlorides, P and the bases on plasma obtained from blood after the addition of lithium citrate. There is the possibility that the addition of this salt to the blood has some effect upon ionic distribution between the cells and plasma.

Eight of the 11 animals used by Swingle and Eisenman were under urethane anesthesia at the time of removal of the blood sample.

Swingle used blood from normal animals for only sugar determinations. The blood for estimation of the other constituents was obtained from cats after removal of one adrenal. In a series of such animals we have found the blood constituents to lie near the outer limits of normal, with a decided tendency toward a shift in the direction of the change found in adrenal insufficiency. In individual cases there was found a definite alteration from normal in the blood constituents. The removal of half the adrenal cortical tissue in some cases may affect the organism to some extent, even though a sufficient amount remains to maintain life and most animals appear to be healthy.

In two partly grown cats and seven kittens we found that removal of one adrenal was followed some weeks later by the typical symptoms of adrenal insufficiency with subsequent death. The remaining adrenal showed depletion of the cortical lipoids. Elliot and Tuckett (29) first pointed out the susceptibility of kittens to partial adrenal removal.

The concept of adrenal insufficiency occurring in spite of the presence of active cortical cells, we believe, is the only justification for the use, as test objects, of animals, for example, the rat, that frequently have accessory cortical tissue. The removal of their glands produces a partial insufficiency. Although they may be normal as far as outward appearances are concerned, this might be due to a readjustment of the endocrine balance with some adrenal insufficiency still present. Their subsequent survival would then depend upon other factors.

The marked changes we have obtained in adrenal insufficiency agree well with those previously reported by other investigators (Table 4). Although there are slight differences in the degree of change, there is substantial agreement that in adrenal insufficiency there is present nitrogen retention, a disturbance of CHO metabolism and an acidosis with loss of both chlorides and base from the blood. In disagreement with Kisch (30) we have found no great change in serum calcium. Moreover, we have found a much greater increase in serum potassium than has previously been reported in cats, although Hastings and Compere have reported a rise in the serum K of adrenalectomized dogs. This rise in serum K and the drop in Na might be overlooked if the changes in the level of base in the blood were followed only by determination of total base.

At this time we cannot say whether the increase in serum K is a compensatory measure to maintain an ionic constant following the loss of sodium, or whether it indicates increased cellular membrane permeability with inadequate excretion through the kidneys. The terminal disturbance of carbohydrate metabolism as shown by the drop in blood sugar might also be considered in evaluating the factors responsible for an increase in serum K.

The lowered blood sugars in experimental adrenal insufficiency and in Addison's disease have frequently been attributed to loss of adrenin by removal of the chromaffine tissue, but Wyman (12) and Zwemer, Smith and Shirley (31) have presented evidence that a small amount of active adrenal cortex will prevent the sugar drop. Further evidence of the effect of the adrenal cortex on blood sugar is shown by a prolonged increase in blood sugar after injections of our extract. Britten and Silvette have recently emphasized the importance of the adrenal cortex in carbohydrate metabolism.

It is not necessary to postulate an unknown toxin to explain the picture of adrenal insufficiency. Chemically, the syndrome is essentially a disturbance of salt and water metabolism. The acidosis has been demonstrated by changes in the blood to be due not to an accumulation of acid but to actual depletion of base.

Gamble and his co-workers (32, 33, 34) have shown that depletion of base in the organism is accompanied by loss of water and conversely that reduction of the water content of the body leads to the excretion of an equivalent quantity of base.

This loss of water is evidenced clinically by the loss of weight and general dehydration of the adrenalectomized animal, and by the increase in total blood solids and decrease in plasma volume. The Na drop and K rise may also indicate that the water loss is from the extracellular fluids, with some replacement by intracellular fluid.

Oliguria consequent upon the dehydration might account for the nitrogen and phosphate retention, or the high NPN (of which the greater part is urea) could be due to a failure of the base-sparing mechanism of NH_3 formation.

Injections of adrenal cortical extract seem to enable the body to bring about a more favorable distribution of salt and water, the rapid gains in body weight being due to a retention of water and Na (cf. Cat L50). Metabolism in general is then improved, since the body fluids, with the approach of normal ionic distribution, would be better able to perform their proper functions.

SUMMARY

1. An analysis is given showing the chronology of the blood chemical changes occurring in adrenal insufficiency in approximately 45 cats. This was a necessary antecedant to the selection of a chemical criterion of adrenal insufficiency. As a control, the values per cent of many of the constituents in the blood of approximately 75 normal cats were determined (Table 1), and compared with the findings of other investigators (Table 2). The control observations were made during the same period of time and under identical conditions with the analyses of adrenal insufficiency and of the changes following treatment.

2. Adrenalectomy was found to be followed by an early loss of Na, which is reflected in a lowered CO_2 capacity, and an increase in K and NPN. Lower blood sugar and Cl and higher P and Mg values also appear before death from adrenal insufficiency. We failed to find important changes in Ca (Table 3). The results of other similar investigations are given (Table 4) and discussed briefly.

3. Chemically, the syndrome of adrenal insufficiency seems to be a disturbance of salt and water metabolism, with loss of water and depletion of sodium and blood chloride, together with an increase of potassium in the blood.

4. Injection of an extract of the adrenal cortex into adrenalectomized animals was found to bring about a reversal of the chemical changes occurring in adrenal insufficiency in addition to affecting the observable symptoms favorably.

5. The suggestion is made that the adrenal cortex secretes something which enables the cells to metabolize salts and water.

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BIOCHEMICAL STUDIES ON THE MALE HORMONE AS OBTAINED FROM URINE*†

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The testicular hormone obtained from human male urine has been the subject of investigation by many workers, notably Funk, Harrow, and Lejwa (1, 2, 3), McCullagh, McCullagh, and Hicken (4), Dodds, Greenwood, and Gallimore (5, 6) and Butenandt (7). With the exception of the last mentioned author, the extracts described by these workers have been rather crude compared to the relatively high purity of the bull testis extracts described by the present authors. The purpose of this communication is to present a method for the extraction and purification of the comb-growth-stimulating substance found in human male urine and to compare the chemical and physiological properties of this substance with the extracts from bull testis.

The urine is acidified with sulfuric acid within two hours after collection to approximately 1 per cent by volume. After filtering through cloth to remove foreign particles, the urine is extracted with benzene in a continuous extractor as shown in Figure 1. When a sufficient quantity has been extracted, the benzene is distilled off, the residue dissolved in ethyl ether and extracted with 10 per cent aqueous NaOH until the alkali layer is no longer colored. The solids removed by the base represent approximately 90 per cent of the total weight of the solids obtained in the benzene extract. No activity is removed in the alkali as reported by Dingemanse (8) but contrary to the findings of Funk and Harrow (9). The ether layer, after being washed until the last traces of alkali are removed, is exaporated to dryness. The residue, when assayed according to our new procedure, shows approximately one standard bird unit per mgm. The details of our assay-technique are reported elsewhere; however, it should be noted here that the important features are as follows: with each unknown or series of unknowns injected into at least 10 and preferably 20 birds, a standard preparation is assayed simultaneously. The comb-growth on the unknowns is calculated in terms of the standard dilution according to the characteristic curve. The result is expressed in standard bird units, i.e., the dose which is equivalent to 1 cc. of our standard preparation. This method is absolutely necessary for comparable quantitative results over an

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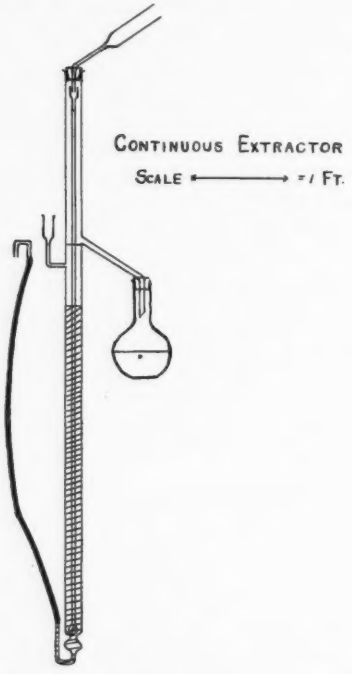
extended time since light is so important a factor in the final growth with any given dilution (10).

The active extract after alkali treatment is in the form of a dark red heavy oil which is seemingly non-toxic in the bird and mammal. Numerous attempts to purify this extract by the usual solvent partitions and precipitations resulted in failure until we resorted to fractional distillation.



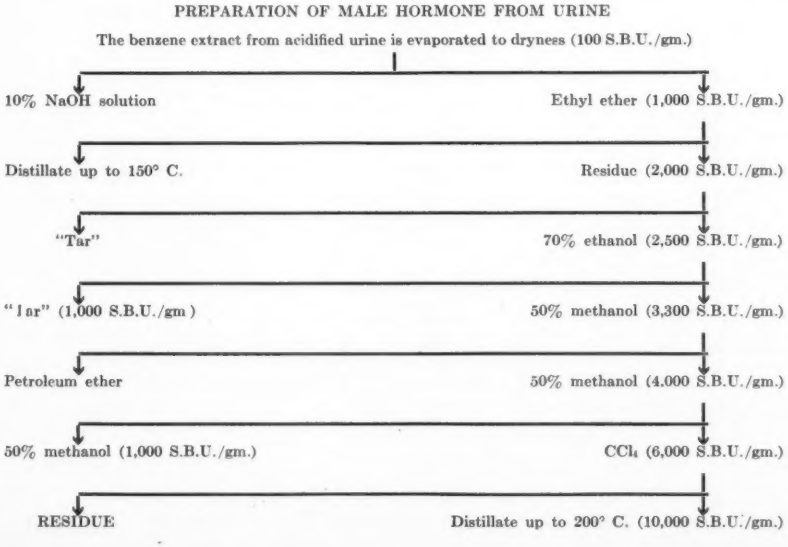
When the material is subjected to a high vacuum of from 0.1 to 0.01 micron of mercury, distillation begins at about 50° C. and a pale yellow mobile oil is obtained up to about 100° C. From 100° to 150° a heavier red oil appears in the distillate which solidifies at room temperature. This distillate up to 150° C. contains no activity and removes about 50 per cent of the solids. The residue is extrated with boiling 95 per cent ethanol and filtered to remove the products of pyrolysis encountered in the course of distillation. The activity of the residue is now about one standard bird unit per 0.5 mgm.

Solvent purification may now be made with favorable results. The residue from distillation dissolved in ethanol is diluted with water to 70 per cent ethanol by volume and centrifuged. The precipitated tar is washed twice with small volumes of 60 per cent ethanol and the washings added to the mother liquor. The tar is practically inert biologically and represents about 20 per cent of the solids. The mother liquor is evapo-

rated to dryness and dissolved in methyl alcohol. Water is added to 50 per cent by volume and the extract is again centrifuged. After the precipitate is washed with small volumes of 50 per cent methanol, the mother liquors are combined and extracted with 40°-60° petrol ether. The precipitated tar and the petrol-soluble fraction represent 50 per cent of the solids and contain usually about 20 per cent of the activity. These figures can, of course, be varied depending upon the volume of solvent used for washing the precipitate from 50 per cent methanol. The material at this stage assays about one standard bird unit per 0.25 mgm.

The activity, now dissolved in 50 per cent methanol, is removed by extraction with small portions of carbon tetrachloride. About 60 per cent of the solids and about 90 per cent of the activity are taken up by the carbon tetrachloride. The standard bird unit at this stage represents about 0.17 mgm. of solid. The material at this stage will yield the oxime crystals described by Butenandt. We were in a few trials unable to obtain them from less highly purified fractions. This point, however, merits further investigation.

The extract may now be subjected to further purification by high vacuum distillation. The distillate obtained between 150° and 200° C. contains the bulk of the activity. The yield of solids varies from 30 per cent to 60 per cent due principally to the difficulty of controlling distillation with the small amounts of material usually available at this stage. The yield in activity is about 80 to 90 per cent. The standard bird unit represents in the neighborhood of 0.1 mgm. or less. A flow sheet presents graphically the method of purification up to this stage of activity. The


PREPARATION OF MALE HORMONE FROM URINE
The benzene extract from acidified urine is evaporated to dryness (100 S.B.U./gm.)
10% NaOH solution
Ethyl ether (1,000 S.B.U./gm.)
Distillate up to 150° C.
Residue (2,000 S.B.U./gm.)
"Tar"
70% ethanol (2,500 S.B.U./gm.)
"Tar" (1,000 S.B.U./gm)
50% methanol (3,300 S.B.U./gm.)
Petroleum ether
50% methanol (4,000 S.B.U./gm.)
50% methanol (1,000 S.B.U./gm.)
CCl_4 (6,000 S.B.U./gm.)
RESIDUE
Distillate up to 200° C. (10,000 S.B.U./gm.)

final product is not as pure as our best bull testis preparations of which the standard bird unit represents less than 0.01 mgm. of solid.

Thus far we have discussed the biological action of this urinary extract from its effect upon the capon comb. The question naturally arises then: does this extract produce all the biological phenomena associated with testis extracts? We believe that our results throw some light upon this problem.

The mammalian tests elaborated by C. R. Moore and the present authors have recently been studied from a quantitative standpoint by Hansen (11). At our suggestion Doctor Hansen was kind enough to assay simultaneously three different dilutions of urinary extract and to compare them with testis preparations of similar potency as determined by previous assays on capons. The dilutions used were furnished to him as unknowns. The assay showed identical results at all three levels of response of the prostate and seminal vesicle cytology. This result has more recently been confirmd in Professor Moore's laboratory by using more highly purified urinary extracts.

It is known from the work of Reese (12) that the "castration cells" in the anterior hypophysis may be restored to normal condition by both testis extract and urinary extract prepared as demonstrated. It is desirable, however, to compare the degree of response obtained by this criterion when equal doses, in terms of bird units, of urinary and testis extracts are administered. This has been studied for us by Doctor Nelson in Moore's laboratory. He reports the same degree of repair with equal dosage whether the extract be from urine or from bull testis. It is a pleasure to acknowledge the friendly cooperation of our colleagues from the Department of Zoology.

Another criterion available for comparing the biological action of the two extracts is the effect upon the plumage of the Sebright capon. Doctor Domm and the present authors (13) have shown that extracts of testis cause the regenerating feather to revert to the type characteristic of the uncastrated male. We have likewise noted that the same dosage in bird units of a urinary preparation produces approximately the same result. This problem is still under investigation.

From the series of different reactions mentioned above we are justified in concluding that the biological activity of the two extracts is probably identical. It is an important problem to study as many and as varied reactions as can be accurately compared to discover whether there is any significant difference between the testis extracts and the urinary product. This becomes of still greater importance in view of our studies on the chemical properties of the two extracts.

The extracts from testis and urine are so dissimilar as regards number and character of impurities present in the two products that chemical comparison is rather risky. This must be borne in mind in comparing our boiling point temperature with testis extracts. Laqueur and co-workers

(14) give the boiling point for testis extracts as 80° to 90° C. at 0.01 mm. Hg. Our own results lead us to believe a higher figure is more nearly correct, yet the difference should not be considered serious in view of possible differences in purity. The boiling point of the urinary product between 150° and 200° is somewhat higher but here again we cannot disregard the influence of the relatively large amount of inert material present. Both urinary and testis products seem to be inactivated by acetylation and oxime formation.

The behavior of the two extracts toward alkaline hydrolysis is, however, significantly different. We have demonstrated that the testis extracts are relatively easily destroyed by boiling NaOH (15). The urinary extracts, on the contrary, are not at all affected by the same or longer treatment. When extracts from both sources are combined and treated with alkali the amount of activity lost corresponds to the amount of testis extract added. This last experiment proves that the lability and stability are properties of the respective compounds and not the result of impurities present in the extract. This important chemical difference between two substances of identical biological character should be an added stimulus to the isolation of the active constituent of bull testis and its comparison with the pure product from urine. Only this comparison can settle the problem adequately.

SUMMARY

Methods for purification of the male hormone from human urine have been described.

The product obtained is thermostable, distilling between 150° and 200° C. at 0.01 micron Hg. pressure.

The results obtained thus far lead us to believe that the active principle from urine is biologically identical with the active component of bull testis extracts.

The behavior of the two extracts toward boiling alkali, however, compel us to believe they are chemically distinct compounds.

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THE EFFECT OF PREGNANCY URINE EXTRACT AND OVARIAN FOLLICULAR HORMONE ON HYPERTHYROIDISM

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Clinical recognition of the importance of ovarian function in thyroid disease led us to try the effect of pregnancy urine extract containing sex gland-stimulating hormone on the course of hyperthyroidism. The preparation used was Antuitrin-S, generously furnished by Dr. E. A. Sharp of Parke, Davis & Company. This corresponds to the Prolan solution of Zondek (1) and has a strong luteinization effect on the ovaries of small animals when large doses are given, but is reported to have a follicle-ripening effect in such experiments when given in high dilutions. Its chief clinical use at present is in the control of menstrual disorders (2). Geist (3) reports that he found at operation no ovarian change in 22 patients who had been treated for two weeks with anterior luteinizing hormone. Recently Mandelstamm (4) found at operation, along with other effects, cystic follicles in the ovaries of patients treated with Prolan but no evidence of luteinization. The solution used by us contains no thyreotropic anterior pituitary substance judged by histologic and metabolic studies, nor does it in luteinizing doses inhibit the action of pituitary extracts containing the thyreotropic hormone (5). In view of these negative results the apparent influence on clinical hyperthyroidism here reported is without physiological explanation.

MATERIAL AND PROCEDURE

The patients observed in this study were in attendance at the Northwestern University Clinic. They were all in financial and social distress. No iodine bearing medication was given. Additional food, chiefly milk, was sometimes available, and increasing amounts of rest were urged. Nevertheless, no substantial change in activity or social status was made. Metabolic rate determinations on these patients were made every two weeks. These are charted in Figures 1 and 2. After the diagnosis of hyperthyroidism was established pregnancy urine extract (Antuitrin-S) was given three times a week, usually by a visiting nurse sent to the patient's home. One or two cubic centimeters, standardized as 100 rat units per cubic centimeter, were given intramuscularly. The medication was given either during the fortnight preceding menses, or that following it, or continuously in all cases beyond the menopause and in some of those menstruating. The injections were discontinued when the metabolic rate fell below +10 per cent.

RESULTS

There is a definite division of the patients according to the effect of the pregnancy urine extract on the course of hyperthyroidism. The group unaffected contains women with failing ovarian follicular growth, that is, those at or beyond the menopause; the group benefited includes those with active ovarian follicle development.

Figure 1 indicates the metabolism measurements of three women at or beyond the menopause, and one boy of 17. The trend of the tests in this group is sustained throughout the period of observation. They were likewise not benefited clinically.

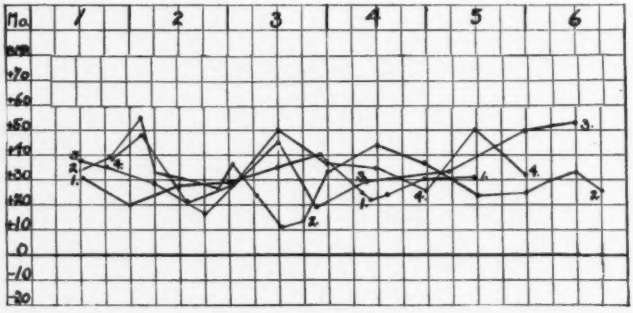

Figure 1. Basal metabolic rate readings of three women, aged 45, 46, and 53, and a boy aged 17. Treatment continued throughout the period of six months.

Figure 2 indicates the metabolic rate readings in five younger women from 17 to 34 years of age, all having regular menses. Of these five, four have been distinctly benefited. There has been in addition to the fall of metabolic rate indicated, decrease in hyperthyroid symptoms and gain in weight. The fifth subject showed a definite period of initial improvement

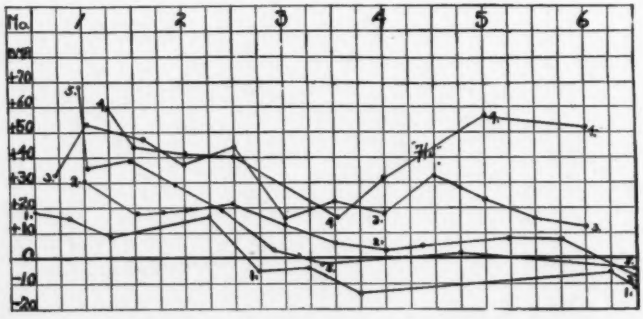

Figure 2. Basal metabolic rate readings covering six months, of five women aged 17, 19, 25, 33, and 34. Treatment discontinued in Nos. 1, 2, and 5, when the basal metabolic rate dropped below +10 per cent; No. 3 is still under treatment; No. 4 is to have a second course.

up to the end of the third month only to relapse after an upper respiratory tract infection. The most striking case is that of a young Italian girl —Figure 2, No. 5—of 17, with hyperthyroidism of about a year's duration. The metabolic rate fell from +38 per cent to 0 in six weeks, with an associated gain in weight of 18 pounds. Medication was then discontinued and the metabolic rate has remained normal for six months. In this case the pregnancy urine extract (Antuitrin-S) was given in the post-menstrual or follicle-ripening fortnight; the follicular hormone (Theelin) in the premenstrual or corpus luteum fortnight. No changes in menstruation of magnitude sufficient to indicate definitely changed ovarian function occurred in any of these cases.

DISCUSSION

Czoniczer and Kleiner (6) reported the effect of anterior pituitary lobe extract in three hyperthyroid patients treated from two to four weeks with Prähormon, a preparation of Prolan, with a lowering of metabolism from an average of +80 to +40 per cent. Two of these patients had had ioidine without benefit. Falta and Högler (7), in a review of the anterior pituitary hormone, report two cases of Basedow's disease treated with Prähormon in which the metabolic rates fell from +47 to +28 per cent and from +74 to +61 per cent. They also state that there is an acute effect, the metabolism being lower one hour after injection, and that Prähormon also lowers the metabolic rate raised by thyroxin administration. Bernhardt (8) states that the action of Prolan is to lower increased metabolism and to raise depressed metabolism. Koehler (9), in a miscellaneous group of cases, treated one subject of Basedow's disease with Prolan injections every second day. In six weeks the metabolic rate fell from +43 to +24 per cent. Anselmino and Hoffmann (10) report the preparation of an anti-thyroid substance from blood and tissues which nullifies the action of thyroxin and the thyreotropic anterior pituitary hormone. According to our studies (5), Antuitrin-S does not have this effect. Aron (11) reports the inhibition of pituitary thyroid stimulation in guinea pigs by massive doses of folliculin. It seems likely that the apparent therapeutic action of pregnancy urine extract in hyperthyroidism may be due to its primary effect on the ovary which under its influence inhibits the thyroid. The striking contrast of result in the two age groups, the excellent result in the youngest woman, and the failure in the boy of the same age, suggest an ovarian action.

SUMMARY

Three patients of menopausal age and a boy of 17 years suffering from hyperthyroidism were not benefited by intramuscular injections of pregnancy urine extract. Four of five women younger than menopausal age were benefited by such treatment. The most striking improvement was in a moderately severe hyperthyroidism in a girl of 17, who was given preg-

nancy urine extract (Antuitrin-S) after, and ovarian follicular hormone (Theelin), before menses.

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SOME ENDOCRINE OBSERVATIONS ON ADVANCED OSSIFICATION IN CHILDREN

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Accelerated bone age is of equal clinical significance with retarded bone age. Few cases of advanced ossification have been reported. This condition occurs in children with sex precocity, from tumor or hyperplasia of the ovaries, adrenal cortex, pineal gland or testes (in order of importance and frequency). Retarted bone age generally is conceded to indicate hypothyroidism. Reasoning by the analogy that childhood myxedema almost always is characterized by retarded bone age, hyperthyroidism and long continued thyroid administration may be considered to be causative factors in accelerated ossification. It is conceivable that an excessive amount of anterior pituitary growth hormone might accelerate ossification; however, the preadolescent hyperpituitary state of gigantism is not associated with advanced ossification.

Roentgenologists have noted in Indian (1) and Egyptian (2) females complete epiphyseal closure three to five years in advance of that of American and European females. In recent years in the University of California Hospital Clinic several cases of premature complete epiphyseal closure have been observed. One boy, aged three and a half years, with pubertas praecox, had an epiphyseal bone age of 8 plus years. Long and Campbell (3) reported markedly accelerated bone age in a group of idiots.

Advanced ossification accompanied by increased height was discovered in 22 girls under observation for obesity in our Ductless Gland Clinic. Of this group, 17 had an early onset of menses and premature development of secondard sex characteristics; three, while not yet at the menarche, had well developed sex characteristics; and two had normal sex development for their age.

Nationality did not seem to be a significant factor in this condition; most of our group were of North European stock; a few were of Latin origin.

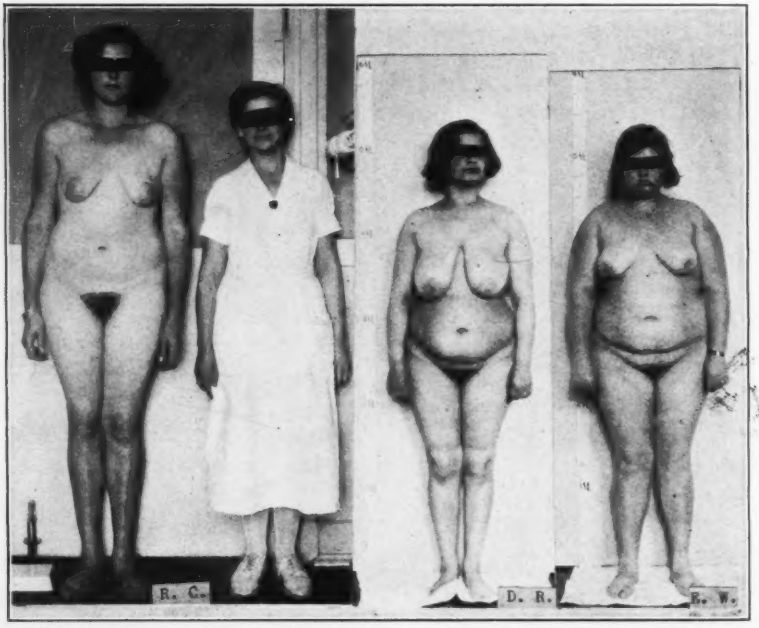
The average age for the appearance of menses in this group was 11 years and 2 months. V. M., at 10½ years, and E. B., at 8½ years, had not yet menstruated but their bone epiphyses were so well developed that the cases are included in the study. Novak (4) states that in this country Englemann reported an average age of 13.9 years for the normal onset of menses, Emmett, 14 years and 2 months, and Currier, 14 years and 6 months. Englemann has noted that first generation American girls, regardless of race, have the menarche at the same age, 13.9 years. as do girls

having many generations of American ancestors. The European menarche of 15½ years may indicate precocity in the American girl.

The obesity and overgrowth in height preceded premature appearance of menses often by many years. It would be unlikely that gigantism would result from early growth in height because advanced ossification leads to premature closure of the epiphyses. L. T. had a normal height for her 12½ years, yet her bone age was 18 years, so that she had only about two years of bone age in which to complete her growth.

The axillary, girdle, and thigh distribution of excess fat resembled that of hypopituitarism, and their pudgy hands suggested the same condition. The hair, skin, tongue, and teeth were normal, as also were the pulse, blood pressure, and hirsuties.

In five of the cases the external genitalia were of normal adult development. Rectal examination of the internal genitalia in 14 cases showed a slightly large uterus in one and a small uterus in five. A possible ovarian cyst was felt in one case. Otherwise, the physical examination gave normal findings.



Random selection, illustrating type of patient. Note the height, development and obesity. R. C. 13½ yrs., D. R. 14 yrs., and E. W. 13½ yrs.

LABORATORY DATA

The following is a presentation of additional findings in this group of cases.

The range of precocious epiphyseal development or difference between chronological and bone age was from 2 to 11 years. These bone ages average 3½ years beyond the chronological age, which is beyond normal limits. Most of the girls

were tall for their ages. The span measurement was just short of the height, except for H. D., who had a span of 4½ inches more than her height. Measurements of the lower and upper part of the body were approximately equal in all except E. B., whose upper measurement was 23½ inches; lower, 32 inches; height 55½ inches; and span, 57½ inches.

The sella was slightly large in three cases and of normal size in nine.

Glucose tolerance tests were made in 10 subjects and only three were outside the normal range. E. L., E. W., and L. T. had excellent tolerance.

The basal metabolic rate in 12 girls ranged from minus 14 per cent to minus 32 per cent, one other had a basal rate of minus 9.5 per cent. Most of the plus readings did not reach 10 per cent on the Benedict-Baker-Henry Standards for 12 to 17-year-old girls. The highest rate was plus 9.5 per cent.

The intelligence quotients averaged between 85 and 100; the highest was 123 and the lowest 53. Mental precocity did not accompany the precocious physical development.

Four attempts to determine sex maturity hormone in the urine by the Zondek-Aschheim test gave negative results as did also two trials for growth hormone.

COMMENT

We have not discovered a syndrome in the boys who have been under our observation which is comparable with that of these girls. The greater number of boys who were tall for their age and conspicuously obese were of normal sex development or evidenced retardation of sex development. Routine bone age studies were not made for the boys' group. Many of them had a depressed basal metabolic rate. The presence of the syndrome in the girls suggests that ovarian function may be a factor.

Excessive height for age and possibly advanced epiphyseal development clinically are suggestive of excess growth hormone from the anterior pituitary. The somewhat early appearance of the menarche might be attributed either to ovarian hyperplasia or to an excess of sex-provoking hormone from the anterior lobe of the pituitary gland.

The pituitary sex hormone ordinarily would not be found in the urine of the normal female adolescent or adult, so that its absence in our cases is to be expected. The growth hormone, although present in excess in the body, would not be expected to be excreted in the urine because of its utilization for linear growth. The growth hormone occasionally is found in the urine of the adult acromegalic.

The supposition that a large sella turcica is indicative of excessive secretion of the gland is overruled by the findings of nine small sellae in this series. However, the size of a ductless gland is not a criterion of its ability to secrete.

The three high glucose tolerance curves are suggestive of pituitary hormone deficiency, although mild hyperinsulinism may have been a factor.

The low basal metabolic rates were not accompanied by signs of thyroid deficiency. They have been found in pituitary deficiency.

It is questionable whether calculations of the basal metabolic rates are justified on the juvenile standard used, because the girls were skeletally and sexually adult. The increased height and advanced ossification preclude the possibility of attributing the syndrome to primary preadolescent

Initial	L. T.	E. W.	S. M.	L. B.	M. O.	V. M.	E. B.	B. S.	E. R.	C. W.	R. v W.
Nationality	Mexican American		French German American	American				German American	Portuguese American	American	Holland French German American
Age — Chronological years	12 5/12	13 1/12	11 11/12	11	13 11/12	10 6/12	8 6/12	12 2/12	14 11/12	14	14 3/12
Age — Bone	19 plus	18 plus	15 to 16	13 plus	17 to 18		11 to 14	16 to 18	20	16 to 17	15 to 16
Sella turcica	Normal	Small	Large	Small		Very small	Large	Normal	Normal	Normal	Normal
Height, inches	54 3/4	63	61 1/4	58	59 1/2	55 1/2	55 1/2	66	60	66	61
Type	Normal	Tall	Tall	Tall	Normal	Normal	Tall	Tall	Normal	Tall	Normal
Span	56	63	60 1/4				57 1/2	63	58 1/4		
Upper	27 1/4	31 1/2	30 1/2		30		23 1/2	32	30 1/4		
Lower	27 1/2	31 1/2	30 3/4		29 1/2		32	34	29 3/4		
Weight — Actual, lbs.	106	204	152	146	106	108	110	187	146	178	145 1/2
Weight — Ideal, lbs.	79	106	102	86	92	74	73	117	100	122	105
Obesity—Onset, years	8	Infancy	0–2	3		8	4	1	14	10	8
Menses—Onset, years	11	11 3/12	10 8/12	None; early secondary sex characteristics				10	12 3/12	10 10/12	11 10/12
Genitalia — External	Normal	Extremely well developed	Normal	Normal adolescent	Extremely well developed	Normal	Normal (no hirsute)	Adult type with large clitoris	Normal	Normal	Normal
Genitalia — Internal	Small uterus	? Small uterus	Negative	Normal	? Large uterus		Negative	Normal	Normal		
Intelligence quotient	98	123			? Above 100	85		96	103	105	117
Basal metabolic	7.2% plus	32% minus	26.2% minus	32% minus	9 5% minus	6.3% minus	6.5% plus	36% minus	3.5% plus	18.5% minus	14 2% minus
Glucose Tolerance, MGS. % fasting, ½–1–1½–2	80–101–86–87	81–95–97–94		80–89–131–69–76	75–131–117–107			89–166–98–74 no glycosuria	98–191–165–82–119. Glycosuria at 1 and 2 hours		
Hormone Test — Growth											
Hormone Test — Maturity	Negative				Negative			Negative			

Initial	M. L.	E. M.	H. W.	M. M.	A. H.	R. B.	D. R.	E. L.	H. McC.	R. C.	E. G.
Nationality	German American		Scotch Irish Welsh American				Italian American	Italian American	Irish American		Italian American
Age — Chronological years	15	14	14 5/12	12 8/12	12 3/12	14	14	14	13 1/2	13 11/12	13
Age — Bone	18 to 20	18	16–17		17–18	17	18 plus	17	18	22 to 25	18
Sella turcica	Slightly large	Small	Small		Small			Normal	Normal	Normal	Normal
Height, inches	60 1/2	62	65 3/4	65	60 1/2		63	65 1/4	63 3/4	70.5	65
Type	Normal	Slightly tall	Normal	Tall	Tall	Tall	Tall	Slightly tall	Tall	Tall	Tall
Span	60	59 1/2	70					67	63	75.5	
Upper	31	31 1/2	33 1/4		29 1/8		33	32 3/4	31 1/2	32.0	
Lower	29 1/2	30 1/2	32 1/2		29 1/8		30	32 1/2	31 1/2	38.5	
Weight — Actual, lbs.	179	176	155	124	110	137	155	227	169	206	221
Weight — Ideal, lbs.	105	110	112	120	85		112	120	110	135	120
Obesity—Onset, years	6	? 1 to 2 years						4 years	9	? at 3 to 4 years	11 years
Menses—Onset, years	11	12	12 10/12	11 3/12	9	11	12	12	10 10/12	11	11
Genitalia — External	Hypertrophic labia				Well developed labia; clitoris negative	Normal			Normal	Normal oligo- and amenorrhea at times	Normal
Genitalia — Internal	?Infantile pelvis ? Small uterus				?Ovarian cyst	Infantile uterus			? Small uterus	Normal size uterus, etc.	Normal
Intelligence quotient	105	Normal	53	98	100	Normal	95	100–110 normal	95	110	Normal
Basal metabolic	18.1% minus	5% plus	3% plus		0.5% plus	15.5% plus	9.4% plus	30.4% minus	24% minus	20.5% minus	35% minus
Glucose Tolerance, MGS. % fasting, ½–1–1½–2		75–105–129–130						71–101–73–69	80–140–151–118	79–142–102–89 Urine negative	111–147–128–111
Hormone Test — Growth									Negative	Negative	
Hormone Test — Maturity									Negative		

NOTE—Since compiling this table, four other girls with similar findings have been seen and at least four others also would have similar findings if thorough investigation were possible.

hypothyroidism. A somewhat early onset of menses is a not uncommon characteristic of childhood myxedema.

The absence of masculine characteristics such as heterosexual hypertrichosis and an enlarged clitoris makes an adrenal cortical dyscrasia an unlikely cause of the syndrome. Furthermore, girls suffering from hyperplasia or tumor of the adrenal cortex do not menstruate early (with the exception of one girl reported by Bullock and Sequeria, who began at 11 years), nor do mammary glands develop early. The absence of hypertension and a florid, plethoric complexion are also somewhat against an adrenal origin.

Precocious puberty in girls, caused by ovarian tumors, is ordinarily characterized by onset of menstruation in the first six years of life, with precocious development of secondary sexual characteristics. These patients are also likely to be large for their age as children, but because of premature epiphyseal closure become short adults.

A primary anterior lobe hyperpituitarism, involving both growth-stimulating and maturity-provoking hormones, might theoretically account for the rapid growth, advanced ossification, and somewhat early sexual maturity. However, it would be difficult to explain, on this basis, obesity and lowered basal metabolic rate. Unfortunately, pathological material is not available to throw light on any of the possible etiological factors considered. The most that we feel justified in surmising is that we may be dealing with a primary preadolescent pituitary eosinophilism and basophilism with secondary ovarian hyperfunction. It is possible that the reverse order obtains and that ovarian hyperfunction is primary and the anterior pituitary excess, secondary. We are led to emphasize this possibility because we are not aware of a similar syndrome in boys.

SUMMARY

A study is presented of 22 girls, mostly of European stock from 8 to 14 years of age, with marked acceleration of bone age, tall and obese, and some years premature physical and gonadal maturity. There was a definite tendency to a lowered basal metabolism. Physical measurements, examinations of the genitalia, and glucose tolerance tests were uninformative. Tests for growth and sex hormones gave negative results.

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STUDIES IN THE PHYSIOLOGY OF THE PROSTATE GLAND*

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I. Radium Emanation to the Testes of Rats; Effect on Testes and Prostates. This study was undertaken for the purpose of determining (1) the effect of radium emanation on the germinal epithelium and interstitial tissue of the testes and (2) the secondary changes which might occur in the prostates and seminal vesicles.

Method. Healthy adult male rats were chosen. The gold seed containing the radium emanation was inserted aseptically into the scrotum about midway between the testes, and the animals were sacrificed after a specified period of time.

Results. Data for body weight and organ weights on 14 animals so treated are given in Table 1, wherein the animals were sacrificed after gradually increasing intervals of time. It is noted that the maximal destructive effect on the testes was not reached until 38 days and that the prostates did not show a similar decrease in weight. Therefore an additional group of 13 animals was treated in exactly the same manner except that they were all allowed to live 43 days. On these latter animals Group 1, Table 5, gives averages of body weights and milligrams of organ weight per 100 gm. body weight for those endocrine organs closely concerned with the sex mechanism. For comparison, Groups 2 and 3 show similar data on two small groups of control animals. Therefore Table 1 represents an orientation study while Group 1 based thereon represents a critical study. Histological examination and comparison of these averages with those of Groups 2 and 3 revealed the following facts:

1. The germinal epithelium of the testes showed a much greater susceptibility to the radium emanation than was noted in the interstitial tissue.

*This research has been conducted under the auspices of the James W Packard Research Foundation, principally in the Department of Urology, Research Division, of the Cleveland Clinic Foundation. It has been made possible through the constant interest, support, and collaboration of Dr. Wm. E. Lower. The x-ray studies of parts IV and V have been done in collaboration with Dr. U. V. Portmann, Roentgenologist. The Roentgen dosages in part 3 were administered by Mr. F. R. Mautz. In all these studies Mr. W. Kenneth Cuyler has been my constant and able assistant. He prepared and stained all tissues, made several hundred photomicrographs, and assisted in the conduct of many of the animal experiments. Dr. Allen Graham, Pathologist, has given valuable advice from time to time. A constant fresh supply of the luteinizing hormone (Antuitrin-S) was furnished gratis for these studies by Parke, Davis & Co. Professor Anton J. Carlson of the University of Chicago has been counselor and critic. To all these and to others of the Cleveland Clinic Foundation who have in various ways contributed toward the carrying out of these experiments thanks are hereby extended.

2. The injury was progressive and required about six weeks to reach its approximate maximum.

3. There was a progressive increase in the amount of interstitial tissue and cells of Leydig observed after 15 days.

TABLE 1

SERIES 1. RATS WHICH RECEIVED ONE SEED OF APPROXIMATELY 0.22 TO 0.26 MILLICURIES IN THE SCROTUM, KILLED AT GRADUALLY INCREASING INTERVALS

Rat No.	Wt. Gm.	Duration Days	Organ	Left Wt. Gm.	Right Wt. Gm.	Left Mg. Ctg.	Right Mg. Ctg.	Average Right and Left Mg. Ctg.
7	301	1	Testis	1.130	1.080	375	358	366
			Epididymis	.438	.352	140	117	128
			Ven. prost.	.118	.130	39	43	41
8	130	3	Testis	.826	.733	635	563	599
			Epididymis	.181	.166	139	127	133
			Ven. prost.	.040	.029	30	22	26
9	170	6	Testis	.907	.925	527	544	535
			Epididymis	.221	.209	130	123	126
			Ven. prost.	.047	.047	27	27	27
10	162	8	Testis	1.034	.906	638	559	598
			Epididymis	.266	.220	164	135	149
			Ven. prost.	.046	.045	28	27	27
11	177	10	Testis	.836	.790	472	446	459
			Epididymis	.191	.211	107	119	113
			Ven. prost.	.080	.078	45	44	44
12	200	13	Testis	.758	.793	379	396	387
			Epididymis	.228	.217	114	108	111
			Ven. prost.	.120	.101	60	50	55
13	191	15	Testis	.973	.996	509	521	515
			Epididymis	.192	.210	100	110	105
			Ven. prost.	.104	.074	54	38	46
14	182	17	Testis	.772	.720	423	394	408
			Epididymis	.233	.231	127	126	126
			Ven. prost.	All .172		94		47
15	222	22	Testis	.630	.512	283	230	256
			Epididymis	....	.217	....	97	...
			Ven. prost.	.125	.077	56	34	45
16	334	29	Testis	.903	.790	270	236	253
			Epididymis	.274	.252	82	75	78
			Ven. prost.	.350	.278	104	83	93
17	238	38	Testis	....	.364	....	152	...
			Epididymis	.230	.213	96	89	92
			Ven. prost.	.152	.123	63	51	57
18	180	49	Testis	.490	.354	272	196	234
			Epididymis	.235	.235	130	130	130
			Ven. prost.	All .298		105		52
19	234	56	Testis	.440	.416	188	177	182
			Epididymis	.268	.268	114	114	114
			Ven. prost.	.240	.160	102	68	85
20	270	64	Testis	.546	.467	198	172	185
			Epididymis	.213	.217	79	71	75
			Ven. prost.	.214	.240	79	88	83

4. The prostate gland was not observably injured when the testes were thus irradiated with radium emanation to the extent of reducing their weight ratio more than 100 per cent. Apparently in some instances such irradiation of the testes brought about glandular proliferation in the prostate.

Conclusion. A high degree of destruction of the testicular tubules by radium emanation has no detrimental effect on the prostate gland in rats.

This fact indicates that such tubular injury does not impair the production of the male sex hormone.

II. The Effect of Gold-filtered Radium Emanation on the Prostate Gland of Dogs. In this study it was desired to ascertain relatively in dogs the dosage of radium emanation necessary when distributed directly into the prostate gland to effect an appreciable decrease in the circumference of the latter.

TABLE 2

RADIUM EMANATION IN GOLD SEEDS IMPLANTED INTO THE PROSTATE GLANDS OF DOGS

Dog No.	Weight Lbs.	Total Seeds	Total Dose	Days Acted	Lobe Implanted	Initial Circum.	Final Circum.	Per Cent Increase	Per Cent Decrease
			mc.			mm.	mm.		
6	49	2	3.67	54	Left	86	103	19.7	....
7	28	1	1.8	43	Left	60	68	13.3	....
29	49	6	7.0	5*	Left	85	79	....	7.0
30	36	8	11.2	50	Both	67	71	....	6.0
31	20	3	4.2	3*	Left	51	52	....	....
39	21	4	8.0	22*	Left	72	56	....	22.2
40	34	5	10.2	35	Left	95	74	....	22.1
41	32	3	6.0	41	Left	84	68	....	19.0

*Animals died of distemper.

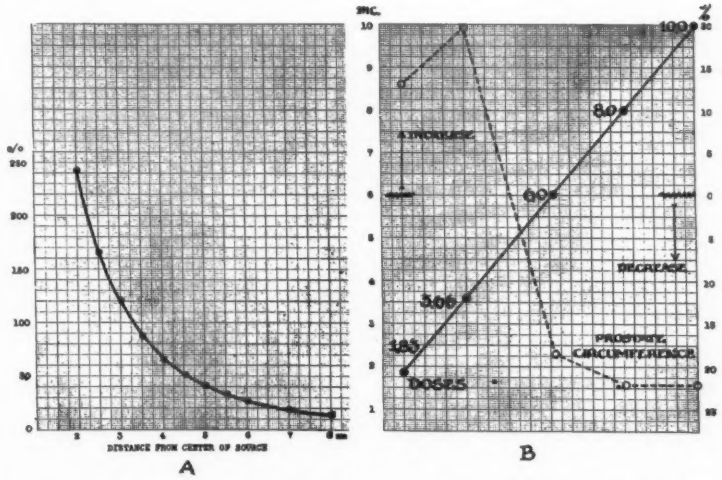

Figure 1. A, the percentage of effectiveness of a radon seed of approximately 2 mc., in the prostate gland of a dog, for distances of 2 to 8 mm. from the source. Note that the 100 per cent necrosis effect extends 3¼ mm.; at 6 mm. the same dose is about one-fourth as effective. B, the percentage increase or decrease in circumference of the prostate gland caused by increasing dosages of gold filtered radium emanation.

Method. Under sodium amytal anesthesia the prostate gland was exposed suprapubically, intraperitoneally, without opening the bladder. The gland was carefully supported from below, avoiding traction, its circumference determined by means of a silk ligature, and immediately thereafter the seeds of radium emanation were implanted.

Results. Table 2 gives each animal's body weight, the dosage of radium emanation administered, the number of days the animal survived after implantation, prostate measurements, and percentage increase or decrease of size of the gland. The relative effects of dosage are shown graphically in figure 1-B. By a histological and statistical study of these data we observed the following facts:

1. The small doses of radium emanation were ineffective.
2. Permanent implants of doses totaling 6, 8, and 10 millicuries, respectively, in three animals were followed by a considerable decrease in size of the prostate gland.
3. In the animal the prostate of which received a total dosage of 10 millicuries of radium emanation there was considerable tubular degeneration of the testes while parts of the prostate gland appeared uninjured.

Conclusions. (1) Small doses of radium emanation are ineffective in reducing the size of the prostate gland of dogs. (2) Symmetrically placed doses of 6 or more millicuries are effective, the result being accomplished by tissue necrosis. (3) To the destructive effects of radium emanation the epithelium of the prostate gland is very much more resistant than is the epithelium of the testis.

III. Roentgen Radiation to the Testes and Prostates of Rats; Effects on Testes and Prostates. The use of Roentgen rays was resorted to in these experiments as a further control on the results obtained with radium emanation to the testes of rats for the reason that the Roentgen rays could be more evenly administered.

Method. Adult albino rats were used. They were anesthetized with sodium amytal and attached by adhesive tape to boards. The x-ray machine was set to give 140 Kv., distance 40 cm., filter 4 mm. of aluminum, rate of administration 40 r. units per minute. The animals were divided into three groups as follows: Series I, rats Nos. 6, 7, 10 and 11, testes only irradiated; Series II, rats Nos. 12, 13, 14, 15, 16 and 17, prostates only irradiated; Series III, rats Nos. 18, 19, 20, 21, 22 and 23, both testes and prostates irradiated.

Results. Table 3 shows in detail the body weight, organ x-rayed, and weight in milligrams organ weight for each 100 grams body weight for the testis, epididymis, and ventral prostate, using in each instance the weight of one paired organ obtained by averaging the weight of both. The animals in Series I and II after irradiation were tested for fertility by mating with females which had been previously tested and found to be fertile. In Series I, Nos. 6 and 7 bore 3 and 2 pups, respectively, following matings 9 and 13 days, respectively, after radiation. No further litters were born to this series. Nos. 10 and 11 were mated 12 and 8 days, respectively, after radiation but were without offspring. The total offspring of Series I was, therefore, 5 pups. In Series II, rat No. 12 mated 5 days after radiation was the father of 6 pups 23 days later, all of which died. No. 13,

mated 4 days after radiation, was without offspring. No. 15, mated 6 days after radiation, was the father of 4 pups 34 days later and 10 more pups 64 days after radiation. No. 16, mated 6 days after radiation, was the father of 8 pups 27 days later. No. 17, mated 11 days after radiation, was the father of 9 pups 33 days later. The total offspring of Series II was, therefore, 37 pups.

By a histological study and comparison of organ weights in the three series the following observations were made:

1. Four times the dosage of Roentgen radiation which caused definite damage to the testicular epithelium when the testes were irradiated

TABLE 3

Rat No.	Wt. Gms.	Part X-Rayed	Mg. Per Centigram (Dose 250 r Units)			Mg. Per Centigram (Dose 500 r Units)			Mg. Per Centigram (Dose 1000 r Units)			Days Post Exp.
			Testes	Epidid.	Prost.	Testes	Epidid.	Prost.	Testes	Epidid	Prost.	
6	392	Testes				212	79	82				70
7	288	Testes							140	67	43	71
10	330	Testes	154	96	84							85
11	289	Testes				388	100	57				85
Charted average.....			154	96	84	300	89	69	140	67	43	
12	217	Prost.				361	112	53				70
13	257	Prost.	206	93	106							77
14	338	Prost.							260	84	59	70
15	281	Prost.	382	137	55							71
16	318	Prost.							374	.....	37	84
17	258	Prost.				385	116	34				71
Charted average.....			294	115	77	373	114	43	317	84	48	
18	172	Pr. & T.	276	143	54							31
19	269	Pr. & T.							104	65	45	68
20	147	Pr. & T.							119	100	56	29
21	135	Pr. & T.				200	137	70				31
22	205	Pr. & T.				123	86	64				30
23	188	Pr. & T.	173	77	31							70
Charted average.....			224	110	42	161	111	67	111	82	50	
General average.....			238	109	65	278	105	60	199	79	48	

apparently caused no essential damage to the prostatic epithelium when the prostates were irradiated.

2. Doses of 500 or more r. units given over the testes of white rats had a high degree of sterilizing effect at least for a period of 80 days.

3. Doses up to 1000 r. units given over the prostates of such animals, with testes shielded, apparently had no sterilizing effect.

Conclusions. (1) A high degree of destruction of the testicular tubules in rats by Roentgen radiation has no detrimental nor atrophic effect on the prostate gland. (2) Doses of Roentgen radiation which have a high degree of sterilizing effect on the testes usually do not impair the production of male hormone as indicated by the weights and histological appearances of the prostates and epididymes.

IV. Roentgen Radiation to the Testes of Rats; Effect on the Amenability of the Testes to Stimulation by the Anterior Pituitary Gonad-stimulating Principle. Inasmuch as the investigations of Ancel and Bouin and numerous others have indicated that the x-rays have no other influence on the testicle than that of depriving the animal of the power of fecundation, the experiments about to be described were undertaken to determine whether the irradiated testis is amenable to stimulation by the gonad-stimulating principle of the anterior pituitary gland as obtained from the urine of pregnant women.

Method. Adult albino rats of fairly uniform size were selected. They were securely fastened to boards, and to reduce complicating factors to a minimum anesthesia was not used. The setting of the x-ray machine was the same as in Study No. III. Fifteen animals were used; they all received the same well-balanced diet and had access to an exerciser. Five of these animals served as untreated controls (Group 4, Table 5). The remaining ten all received the same Roentgen dosage, 300 r. units, to the testes at one seance. Subsequent to radiation, five of these latter ten animals (Group 5, Table 5) received daily for six weeks 5 rat units of Parke, Davis & Co.'s luteinizing hormone, now known as Antuitrin-S, obtained from the urine of pregnant women. The other five irradiated animals served as irradiated controls (Group 6, Table 5). All of the 15 animals were killed at one time six weeks after the radiation of ten of them.

Results. The data referred to in the preceding paragraph represent group averages for body weight and milligrams organ weight per 100 grams body weight for the testes, adrenals, seminal vesicles with adherent dorsal prostates, and the ventral prostates. In each instance the organ weights represent one organ based on the average weight of the two paired organs.

By a histological and statistical study we observed the following facts:

1. Weights of the testes and prostates of the control group No. 4 agree essentially with those of controls in Study I, groups Nos. 2 and 3, so that for practical purposes these weights represent averages for 14 animals.

2. Whereas irradiation of the testes of rats reduced the average weight of these organs to approximately 29 per cent of that of 14 controls, the average weight of the ventral prostates of these animals was only 9 per cent less than that of controls. In such rats even greater differences in

prostatic weight than 9 per cent occur naturally among healthy normal animals.

3. The average weight of the ventral prostates of the animals which were irradiated and also received the gonad-stimulating principle (Group 5) was approximately 70 per cent greater than that of controls and 90 per cent greater than that of the irradiated animals which received no injections of the gonad-stimulating principle.

4. Likewise the average weight of the testes in the treated x-rayed animals (Group 5) was 44 per cent greater than that of the testes of untreated x-rayed animals (Group 6).

5. The average weight of the seminal vesicle and adherent dorsal prostate of the five animals, the testes of which were irradiated (Group 6) was 162 mg., while the average weight of those of 14 controls was 165 mg. These results indicate that irradiation of the testes did not impair the production of the male hormone. Histologically there was apparently no difference in these accessory organs in the irradiated animals and in the controls.

6. The average weight of the seminal vesicles and dorsal prostates of the animals the testes of which were irradiated and which rats also received injections subcutaneously of the gonad-stimulating principle (Group 5), was approximately 93 per cent in excess of that of controls.

Conclusions. (1) Irradiation to the testes of sufficient intensity to be highly destructive to the germinal epithelium apparently does not impair the production of the male sex hormone as evidenced by the weights of the accessory sex glands. (2) In animals so treated both the testes and the accessory sex glands are amenable to stimulation by the gonad-stimulating principle of the anterior pituitary gland.

V. Fractional Roentgen Radiation of the Testes of Dogs. This study on dogs was made as a further control on the two preceding studies on rats, with respect to the effect on the prostate gland of Roentgen radiation to the testes. In these latter experiments the goal has been to give very much larger doses of irradiation by recourse to the fractional method.

Method. The dogs were anesthetized with sodium amytal (60 mg. per kilo body weight) and fastened supine on boards. The machine was set 195 Kv. filter copper .53, aluminum 10 mm., distance 40 cm., time 105 milliampere minutes for each fraction of 400 r. units. Dogs 90 and 93 received four fractions of 200 r. units each at least one week apart. All the other dogs received fractions of 400 r. units each at intervals of at least one week.

Results. Table 4 gives the animal weight, dosage in r. units, number of days from last dose to postmortem examination, circumference of prostate before irradiation and after completion of the study, per cent increase or decrease in prostatic circumference, per cent increase or decrease in ani-

mal's weight from the time of the initial measurement of the prostate to the time of postmortem examination. Attention is called to the fact that in all instances in which the prostate seemed to increase in size following the fractional irradiation of the testes there was at least just as great increase in the body weight of the animal, which would indicate that the

TABLE 4

CHANGES IN THE PROSTATIC CIRCUMFERENCE AND IN BODY WEIGHT OF DOGS FOLLOWING VARIOUS DOSES OF X-RAY IRRADIATION OF THE TESTES

Dog No.	Dose r Units	Days After Last Dose	Circumference of Prostate		Change in Circumference		Initial Body Weight	Per Cent Change in Wt. After Irradiation	
			Initial	Final	Increase Per Cent	Decrease Per Cent	Lbs.	Increase	Decrease
90	800	69	66	88	30		51	31	
93	800	69	99	91		8	44	16	
103	1200	62	50.5	62	22		38	21	
104	1200	62	75	84	12		40	12	
105	1200	62	69	65		5.8	30	0	
108	1600	63	96	85		11.4	65	0	
111	1600	63	97.5	67		30	50		24
91	2000	72	96	77		20	38	21	
94	2000	71	35.5	42.5	19.7		36	50	
84	2000	72	75	68		9.3	31	12	
100	2000	88	100	93		*7	60		
101	2000	98	94.5	85.5		9.5	60		
102	2000	113	70	80	14.3		37	19	

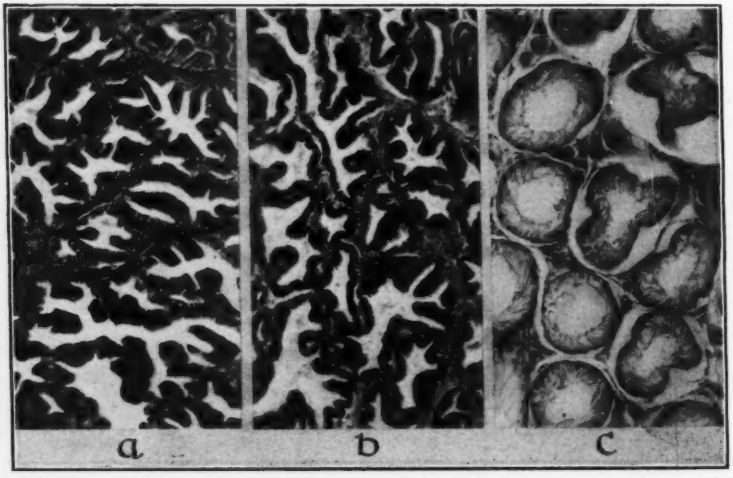

Figure 2. (a) Biopsy of prostate before irradiation of the testes, Dog 84. (b) Same prostate 72 days after the beginning, 44 days after the completion of fractional irradiation to the testes with 2000 r. units. (c) Testis. Note the tubular degeneration and large amount of interstitial tissue.

prostate ratio was essentially undisturbed in such cases. On the contrary, with the very large doses of 2000 r. units to the testes reductions in the circumference of the prostate gland have been only slight even when the destruction of the testicular tubules (evidenced by histological examination) has been profound. A typical example is shown in Fig. 2. Note that the epithelium of the prostate gland appears unchanged as a consequence of the irradiation of the testes.

Conclusion. The dog's prostate gland is probably independent of the testicular tubules for the maintenance of its size, structure, and function, because it reveals no essential change in instances after the tubules of the testes have been almost totally destroyed by irradiation.

VI. The Effect of Cryptorchism on the Pituitary-Testis-Prostate Relationship in Rats, and the Amenability of the Cryptorchid Testis to Stimulation by the Gonad-stimulating Principle of the Pituitary Gland. In these experiments 26 rats rendered artificially cryptorchid survived to the completion of the study.

TABLE 5

SUMMARY OF AVERAGES OF RAT DATA

	Group No.	Study No.	Total Rats	Av. Wt. Gms.	Survived Days	Av. Mg. Organ Wt. per Ctg. Body Wt.				
						Testes	S.Ves.& Dor. Pr.	Vent. Prost.	Adrenal	Pituitary
Radon 0.2 mc. to testes	1	1	13	191	43	268	184	71	8.0	4.5
Controls	2	1	5	196	..	650	208	68	8.3	4.4
Controls	3	1	4	178	..	605	131	64	8.3	3.8
Controls	4	4	5	211	..	668	158	65	6.4	...
X-ray 300 r units (plus GSP 5 units daily)	5	4	5	192	42	269	320	110	8.2	...
X-ray 300 r units to testes (nothing else)	6	4	5	228	42	186	162	58	6.8	...
Cryptorchids	7	6	19	236	35–104	176.7	141.7	61.7	7.2	3.9
Cryptorchids (plus GSP 5 units daily)	8	6	7	187	35	166	301	120	7.7	3.9
Albino rats GSP 5 units daily	9	7	8	176	26–38	759	402	120	9.3	3.9
Black and white rats GSP 5 units daily	10	7	6	124	28	855	533	156	9.5	4.2
Scrubby albinos GSP 10 to 45 units daily	11	7	12	162	28	637	382	118	10.2	4.9

GSP—Gonad-stimulating principle.

Method. Under sodium amytal anesthesia and with aseptic precautions the testes were detached from the scrotum and drawn up into the peritoneal cavity, with the entire blood supply intact, and sutured to the posterior aspect of the anterior abdominal wall in the region of the umbilicus.

Results. Group 7, Table 5, shows averages on 19 untreated cryptorchid animals. For all organs, except the pituitary, the organ weight represents one organ based on the average weight of both. Similar data are given in

Group 8, Table 5, on seven cryptorchid animals that received 5 rat units daily of the extract containing the gonad-stimulating principle of the anterior pituitary gland and were autopsied the day following the 28th intraperitoneal injection. For average control weights on 14 non-cryptorchid animals refer to Groups 2, 3 and 4, Table 5. Figure 3 shows graphically the averages of Groups 7, 8 and 9.

From a histological examination of the organs and comparison of these data the following facts were observed:

1. Artificial cryptorchism, *per se,* did not impair the hormone-producing function of the testis in rats, judged by the weights of the accessory sex glands.

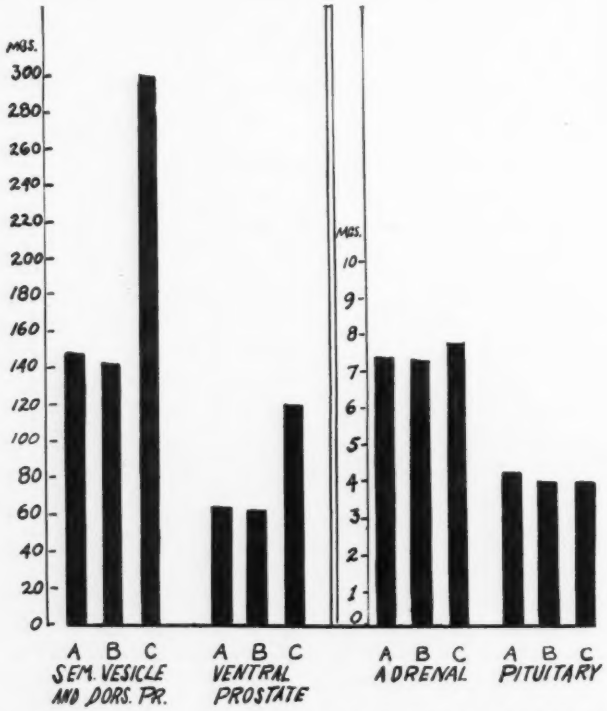

Figure 3. A, controls; B, cryptorchid animals; C, cryptorchid animals injected with the gonad-stimulating principle.

2. The cryptorchid testes were amenable to stimulation by the gonad-stimulating principle of the anterior pituitary gland as contained in an extract of urine from the pregnant women.

3. This stimulation was functional; in our experiments it did not increase the size of the cryptorchid testis as it did the normal testis as shown in the study which follows.

VII. The Effect of the Anterior Pituitary Gonad-stimulating Principle on the Testes, Prostates, Seminal Vesicles, Adrenals, and Pituitary of Male Rats. It has been shown elsewhere in this communication (Study IV) that the gonad-stimulating principle of the anterior pituitary gland as obtained from the urine of pregnant women is capable of profoundly stimulating the testis the tubles of which have been severely damaged by Roentgen irradiation. The purpose of the present study was to determine the effect of similar doses of the gonad-stimulating principle on certain endocrine organ weights of normal rats.

Method. One group of eight young albino rats received intraperitoneally 5 rat units daily as noted in Group 9, Table 5. The minimum number of such daily injections was 26, the maximum 38, prior to autopsy. Another group of six young adult black-and-white male rats received 5 rat units daily for 28 days prior to autopsy (Group 10, Table 5). A third group of 12 very scrubby adult albinos received 28 daily injections prior to autopsy as follows: 4 animals received 10 units daily, 3 animals 15 units daily, 3 animals 30 units daily, and 2 animals 45 units daily (Group 11, Table 5). Average milligrams organ weight per 100 grams body weight for the testis, seminal vesicle with adherent dorsal prostate, ventral prostate, adrenal, and pituitary, are given. On all these organs except the pituitary the weights represent one paired organ based on the average of both.

Results. A statistical study of these data revealed the following facts:

1. Of 14 healthy animals (Groups 9 and 10) which received injections of the gonad-stimulating principle the average weight of the testis was 25 per cent greater than that in 14 controls (Groups 2, 3 and 4).

2. In the 26 animals which received the gonad-stimulating principle the average weight of the seminal vesicles with dorsal prostates was 254 per cent of that of 14 controls, and ventral prostates 193 per cent.

3. The average adrenal weight in the injected animals was approximately 27 per cent greater than that in the 14 controls.

4. There was essentially no change in the average weight of the pituitary gland as compared with that of controls.

Conclusions. By comparison of these results with those in Studies IV and VI we found that the gonad-stimulating principle was about equally effective in normal and cryptorchid animals and only slightly less effective in animals the testes of which had been irradiated, as manifested by the weights of the accessory sex glands.

LITERATURE

In a previous communication Lower and Johnston (1930) reported on the effect of injection of certain strongly germicidal chemical agents directly into the prostate glands of rats and dogs.

I and II. Radium Emanation Compared with Other Forms of Radiation. Delaplace and Rebiere (1929) showed that ergosterol could be converted into vitamin D by weak Roentgen radiation. Maisin, Mund, Pourbaix, and Castille (1930) accomplished the same result by radiation with radium emanation. Crookes (1903) photographed the spectrum of radium emanation and demonstrated in it the presence of the characteristic bands of ultraviolet light. Czarnecki and Jolly (1929) demonstrated that direct exposure of the exposed testis of white rats to ultraviolet radiation from a quartz mercury vapor lamp 30 minutes to two hours resulted in the reduction of the number of spermatozoa present in the testis within one to four weeks. Bissonnette (1931) showed that ordinary white light has a profound stimulating effect on the sexual cycle of male birds (Sturnus vulgaris) inducing spermatogenesis during the quiescent period. He observed that red light was definitely more stimulating than white light, while green light was inhibitory. Aquehon and Robert (1915) produced acceleration of growth of the aerial part of peas, beans, and wheat by subjecting the cultures to the influence of radium emanation produced by 3 mg. of radium screened by lead. A comprehensive review of the literature on the stimulating effect of x-rays was made by Gordon (1930). The great potency of radiant energy is exemplified by such phenomena as phototaxis and photosynthesis in plants, photocatalysis in chemical reactions, especially made use of in photography, and the depression of the leucocyte count following prolonged exposure to gamma rays.

From a review of the results of the above mentioned authors it appears (1) that the long wave lengths of the spectrum stimulate when given in intensities which would be depressing with short waves, or by gamma rays, (2) that radiant energy, in common with other potent toxic agents, has a stimulating and a depressing phase which must be qualified by dosage for the particular wave length involved, and (3) that there is a selective action depending upon the type of protoplasmic tissue radiated. From this fact it may be inferred that while the gonadal epithelium is being depressed by minimal depressant doses of irradiation, more resistant tissues, such as for example the prostatic epithelium, may be stimulated, because for the latter tissue the depressant threshold has not been reached.

Regaud and Blanc (1906) showed that x-rays attack spermatogonia electively in the testes. Regaud and Dominic (1912) in a study on the testis concluded that the effect of x-rays and the gamma rays of radium, suitably filtered, is identical. Regaud (1922) reported complete sterilization of sheep with radium emanation in a dosage of 4.5 millicuries destroyed, and that increase of the period of time during which irradiation was administered, without increasing the dose, greatly favored the sterilizing effectiveness. Bagg (1920) implanted seeds of radium emanation in glass tubes into various tissues, using seeds 2.5 to 4 mm. long by 0.4 mm. thick. In muscle he obtained a necrotic field 9 mm. in dia. using a 0.4 mc. seed, and in the testis of a rat a field of 8 mm. in dia. with a 0.2 mc. seed.

The field of necrosis was about the same for doses of 1 to 5 mc. in single implants.

Duffy (1929) observed that by using a gold seed of 2 mc. instead of a glass seed of 1 mc. twice the amount of gamma radiation was effective with only one-fifth the volume of necrosis.

Quimby and Martin (1929) have experimentally determined the dosages of radon necessary to produce standard effects with various arrangements of implants. They found that in order to radiate a mass of tissue effectively with a group of tubes the range of desired effects for each tube or seed, called the 100 per cent zone, should be calculated and the implants should be so placed that these zones are in contact. If the desired symmetry of distribution is not obtained the sphere irradiated will be smaller than that for which the dosage was intended. For certainty of effects, therefore, they recommend overdosage.

Quimby (1930) has constructed charts from experimental data comparing the bleaching of butter by radon seeds of different strengths and by 1 milligram radium needles for exposures of various durations.

III, IV and V. Effects of X-rays on Gonads. Credit is given to Schönberg (1903) as being the first to study the effects of Roentgen radiation on the testicle. A similar study was made by Frieben (1903), Seldin (1904), Bergonie and Tribondeau, Buschke and Schmidt (1905), Villemin, Roulier, and Regaud and Blanc (1906) and Ancel and Bouin (1907). In reviewing and extending these various studies it was concluded by the latter workers that the x-rays had no other influence on the testicle than that of depriving the animal of the power of fecundation. Zindel (1913) studied the prostates of dogs after x-raying the testicles and concluded that in no instance was there a deviation from the normal prostate. Von Lichtenberg (1922) observed that the Sertoli cells could resist the x-rays relatively long and that they could proliferate within moderate limits. He cites that this fact has been established by Thaler (1906), Buschke, Schmidt, Villemin, and others. Hooker (1925) studied the effect of various doses of Roentgen radiation of the testes on reproduction. Of 19 of the male rats the testes of which received doses of 1 to 3 erythema, 15 were infertile for periods varying from 120 to 240 days after radiation.

Oslund and Bachem (1926) radiated rat testes with doses up to 19 erythema and observed that actively dividing cells (spermatocytes and spermatogonia) were most easily injured, that spermatozoa disappeared for a short time about ten days after exposure, and that Sertoli cells persisted with doses up to 7.5 erythema. They considered that the histological picture was again normal within three months after radiation, which fact attests that the destructiveness of their dosages to the germinal epithelium was not so severe as our doses of 500 and 1000 r. units of hard irradiation.

Ferroud and Regaud (1927), irradiated the testes of 14 rabbits with heavy doses of highly filtered hard Roentgen radiation and concluded that

under the conditions of their experiments it was impossible to obtain in a single irradiation the complete sterilization of the rabbit's testis without producing very grave lesions in the skin and rectum. The same authors (1927) determined by experiments on rabbits that the testes and skin have a very different susceptibility to x-rays, when the dose is fractionated. They observed that doses of 1 to 2 erythema given at one time do not permanently arrest spermatogenesis. If, however, these same doses are given in 5, 10, 20 or 50 fractions at intervals of 10 to 24 hours, the entire treatment lasting 10 to 30 days, the testes of some of the animals are rendered sperm-free without the production of skin lesions. Histological examinations covered the period 22 to 95 days after exposure.

Asdell and Warren (1931) subjected rabbits' spermatozoa to all Roentgen wave lengths of filtered and unfiltered radiation produced at 100 to 200 kilovolts in doses varying from 1 to 11 erythema. Motility studies of the sperm during these experiments gave no indication of any change resulting from irradiation. By artificial insemination of rabbits with such radiated sperm they obtained two litters of apparently normal young rabbits.

Zondek and Aschheim (1926) and Smith and Engle (1927) produced precocious sexual maturity in white rats and mice by anterior pituitary implants. The latter observers could not detect any effect on the reproductive system of normal adult male rats following 15 daily homoimplants, but ten implants in young males resulted in some increase in testis size. Engle (1929) observed that daily pituitary transplants from castrated animals were more potent than those from normal animals in inducing premature sexual maturity. Steinach and Kun (1928) obtained marked stimulation of the accessory reproductive organs of rats by the injection of aqueous extracts of fresh beef hypophysis. Riddle and Flemion (1928) injected extracts of the anterior pituitary gland into immature doves and pigeons causing premature sexual maturity. They considered the testis changes in these birds the most sensitive known test for the sex-maturing principle. Evans and Simpson (1928) concluded that the anterior pituitary gland contains two antagonistic hormones, one necessary for growth, the other for normal development of the gonads, thyroid, and adrenal cortex. They believe that the growth hormone originates in the eosinophilic cells and that the sex autocoid comes from the basophilic cells. Putnam (1929) separated the growth-promoting principle from that causing premature sexual development. Riddle and Braucher (1931) observed that when eggs were incubated by mature pigeons the extremely thin crop wall thickened about 1500 per cent and produced the so-called crop milk. They observed that by injection of an extract of the anterior pituitary gland this thickening and production of crop milk could be made to occur in two-weeks-old squabs of either sex that were receiving crop milk for their nourishment. Riddle and Polhemus (1931), working with pigeons, observed that the response of the testis to injected extracts of the anterior

pituitary gonad-stimulating principle was at least three to five times greater than that of the ovary. Riddle (1931) reported that the daily injection of such extracts brought about as much as 1500 to 2000 per cent increase in the weight of ring dove testes in ten days.

Kallas (1929) showed that sexually immature male rats in parabiosis with similar castrated animals undergo precocious genital development in 8 to 10 days, characterized by increase in the size of the testes and of the accessory sex glands. The pituitary of the castrated animal hypertrophied. If folliculin was administered to a female castrate, the hypophysis retrogressed. Martins (1929) observed that by means of injection of blood from a castrated infantile animal into a normal infantile animal precocious sexual maturity was induced. Zondek and Aschheim (1928) and Zondek (1929) described the preparation, biology, and clinical aspects of hormone from the anterior lobe of the hypophysis which they called prolan, obtained from the urine of pregnant women. They state that the active principle is a very sensitive body, insoluble in most lipoid solvents, and in contrast to folliculin is destroyed by heat, strong acids and alkalies. It is precipitated by such solvents as ether, methyl alcohol, or acetone. From such precipitates the active principle can be purified by redissolving in water. De Jongh (1930) by injecting similar extracts from the urine of pregnant women obtained marked increase in size of the accessory sex organs of young mice 15 days old. The testes of these animals revealed increase in the number of Leydig cells. No effect was observed on the accessory sex organs of castrated mice. Kraus (1930) injected immature male mice with prolan (Aschheim-Zondek) and noted definite stimulation of the male accessory sex glands and marked stimulation of the interstitial cells of the testes. Spermatozoa were not observed to appear any earlier than in the uninjected controls. De Jongh and Laqueur (1931) injected 5 to 6 rat units per day of a preparation from early pregnancy urine into adult male rats, with rough coats, unsuitable for breeding. The testicular weight of these animals increased 50 per cent, the weight of seminal vesicles increased 700 per cent. Evans, Meyer and Simpson (1931) observed that the hormone obtained from the urine of pregnant women (prolan) fails to influence sexual development and function in hypophysectomized animals, and they concluded that either the prolan converts a prohormone into a gonad-stimulating one, or that it converts the growth hormone into a gonad-stimulating one. Claus (1931) obtained a crystalline substance by extraction of various tissues (posterior lobe of hypophysis, epididymis of bull, thyroid gland, adrenal cortex, and fish sperm) which when injected into immature male or female mice induced precocious sexual development. This crystalline subtance was ineffective when injected into spayed female mice.

Moore and Price (1931) injected an extract of pregnancy urine into male rats and observed that the effects on the genital system differed only in degree from those obtained by implantation of fresh pituitary tissue.

Their extract was prepared by Van Dyke and Wallin-Lawrence and by them called hebin. Untreated pregnancy urine was observed by Fels, Steinach and Kun, Engle, Bourg, and Laurent, quoted by Moore and Price, to exert marked stimulation of the accessory reproductive organs.

It must be kept in mind that the criteria of effectiveness in males of a preparation containing the gonad-stimulating principle of the anterior pituitary gland presupposes that the injection of such a substance will stimulate the gonads thereby leading to the overproduction of the male hormone, which in turn will lead to hypertrophy of the accessory sex glands. The indicators, therefore, are the same as for the male hormone. An important difference in the two agents is that the gonad-stimulating principle acts only when at least one of the gonads is present, while the testicular hormone when injected acts in the absence of both gonads. Moore and Gallagher (1929) have adapted the electric ejaculation test, Moore, Price and Gallagher (1930) the rat prostate cytology test, and Moore, Hughes and Gallagher (1930) the seminal vesicle cytology test, as indicators for the male hormone.

Several workers have shown a relationship of the adrenals to the pituitary-gonadal mechanism. Reference has been made to the observation of Evans and Simpson that the anterior pituitary gland is necessary for the normal development of the gonads, thyroid, and adrenal cortex. Guthrie and Enery (1907) had noted that hypertrophy of the adrenal cortex was associated with sexual precocity. McKinley and Fisher (1926) observed testicular enlargement following the feeding of adrenal cortical material. Corey and Britton (1931) induced precocious sexual maturity in infantile male rats by five daily injections of cortico-adrenal extract. Papanicolaou (1931) injected the urine of pregnant cows into premature guinea pigs and observed increased vascularization in the adrenals, thyroids and hypophysis, emphasizing that it was particularly evident in the adrenals.

The gonad-stimulating principle of the anterior pituitary gland in the form of prahormon and prolan has been used by Schapiro (1930) in the treatment of hypogonadism in a series of eighty human patients in the past two years. He reported in 14 of these enlargement of the testes and penis, but not to normal size, and in nine cases complete sexual maturity with existence of spermatozoa and normal sexual function. He emphasized that treatment should be continued a period in excess of five months before pronouncing the results negative.

Collip, Thomson, Browne, McPhail and Williamson (1931) report that acetone extracts of the human placenta contain two active substances apart from ether-soluble oestrin—an alcohol-soluble fraction called by them emmenin, active orally, which causes premature oestrus, and an alcohol-insoluble fraction which is inactive orally, called the A.P.L. (anterior pituitary-like) principle. This principle promotes growth of the prostate and seminal vesicles in male rats and causes premature oestrus in female rats.

Collip (1933) holds the view that the gonad-stimulating principle obtained from the urine of pregnant women has its origin in the placenta. His latest publication on these placental hormones will soon appear in Pfluger's Archives.

Campbell (1932) cites interesting clinical results on women following the administration of the A.P.L. principle.

We know that the gonad-stimulating principle of the pituitary is common to both sexes. Apparently definite proof has not been given that the placenta is not a reservoir for the gonad-stimulating principle originating elsewhere similarly as the liver is a reservoir for glycogen, though the metabolism of glucose is regulated largely by the secretion of the islets of Langerhans.

VI. Cryptorchism. Oslund (1926) states that it appears that germ cells are usually present in the cryptorchid testis and that the hormone is produced in this testis during the absence of mature spermatogonia, spermatocytes, spermatids, and spermatozoa. Martins (1930) showed that in cryptorchid rats there is an increase of circulating anterior pituitary hormones. With a parabiotic normal female plus normal male there was no alteration of the sexual phenomena of the former, but sometimes absence of sexual cycles. On the contrary, in parabiosis of a cryptorchid male plus normal female there were protracted full oestrus stages lasting as long as 42 days. Korenchevsky (1930) observed that in 20 of 23 litters of rats the prostate and seminal vesicles were smaller than those of controls, both relatively and absolutely. The adrenals increased in weight in 7 of the 23 litters of cryptorchids and decreased in the remaining 16 litters. Cryptorchism effected no noticeable change in the hypophysis weights. The duration of his experiments in most of the animals was from ten weeks to six months. Foucin (1931) noted that in the immature testis of the guinea pig rendered cryptorchid the injection of urine from pregnant women caused the interstitial tissue to develop rapidly and that the development of the seminal vesicles ran parallel with that of the interstitial gland, and these organs attained to the full size as in normal animals.

SUMMARY

This report, a study of the physiology of the prostate gland and its hormonal relations, is based on 120 adult rats, average weight approximately 190 grams, and 19 adult dogs.

The average testis weight of 13 white rats was reduced to 42 per cent of that of 14 controls by 0.2 mc. of radon in the scrotum for six weeks. Ventral prostate weights were 110 per cent, seminal vesicle weights 107 per cent.

Radon, approximately 2 and 4 mc., respectively, in the prostates of two dogs, did not arrest growth in six weeks. These glands increased in circumference 13 and 20 per cent, respectively. Doses of 6, 8 and 10 mc.

reduced prostatic circumference 22, 22 and 19 per cent in three dogs in 22, 35 and 41 days, respectively, by necrosis contiguous to the source.

The testes were radiated at intervals of one week in 13 dogs, with total doses of 800 to 2000 r. units. In all, the tubular epithelium was profoundly injured; with 2000 r. units destruction was almost total. Comparison of the prostatic epithelium with that of the corresponding biopsy showed no essential change.

Four fertile adult rats received doses of 250 to 1000 r. units to the testes. Within 60 days their total offspring from fertile females was 5 pups. Five other rats received equivalent doses to the ventral prostates. Their total offspring was 37 pups in 60 days.

The testes of ten adult rats were x-rayed with doses of 300 r. units. After six weeks the average testis weight of five animals was 29 per cent of that of controls, ventral prostate 97 per cent, seminal vesicles 88 per cent. The other five x-rayed rats received five rat units daily of Antuitrin-S. After six weeks the average testis weight was 42 per cent of that of controls, ventral prostate 192 per cent, seminal vesicles 166 per cent.

Cryptorchism was produced surgically in 26 adult rats. The average testis weight of 19 of these was 27 per cent of that of controls, ventral prostate 93 per cent, seminal vesicles 85 per cent. The remaining seven cryptorchid rats received daily injections of 5 rat units each of the Antuitrin-S. The average testis weight was 26 per cent of that of controls, ventral prostate 181 per cent, seminal vesicles 181 per cent.

Eight adult albino rats received 5 rat units daily of the Antuitrin-S for approximately one month. The average testis weight was 118 per cent of that of controls, ventral prostate 181 per cent, seminal vesicles 242 per cent.

Six adult black-and-white rats received exactly the same treatment as the preceding eight rats. Their average testis weight was 133 per cent of that of controls, ventral prostate 236 per cent, seminal vesicles 321 per cent.

Twelve unkempt, scrubby albino rats received from two to nine times the amount of Antuitrin-S as the preceding groups of 7 and 8 rats. Their average testis weight was 100 per cent of that of controls, ventral prostate 180 per cent, seminal vesicles 230 per cent. There was no apparent difference in effect of the two-fold and the nine-fold doses.

Therefore, in rats the gonad-stimulating principle was effective in stimulating the normal testis, the cryptorchid testis, the testis which had been heavily exposed either to radium emanation or x-rays and thereby markedly reduced in size. The effect was in most of these instances a moderate increase in size of the testis, and always a very marked increase in size of the accessory sex glands.

Very heavy Roentgen doses fractionally administered to the testes in dogs failed to produce castration effects on the prostate glands of such animals, indicating that such testes maintain their hormonal function.

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BOOK REVIEWS

THE TIDES OF LIFE: THE ENDOCRINE GLANDS IN BODILY ADJUSTMENT. R. G. Hoskins. W. W. Norton & Company, Inc., New York. 1933. P. 352.

The book, as stated in the preface, represents an attempt to present in brief form the more significant aspects of endocrinology as known today.

DIE PHYSIOLOGIE DER HODENS, Sand, Knud, P. 252. Reprinted from Handbuch der Inneren Sekretion Bd. II, Curt Kabitzsch, Leipzig. 1933.

This work brings up to date in an admirable way our knowledge of the physiology of the testes. After a section devoted to the gross and microscopic anatomy the reproductive function is considered. Next the rôle of the gonad in the control of metabolism, heart-regulation and muscular activity is discussed. Other sections are devoted to: The effects of castration and of testis grafts; the influence of a variety of conditions on the testes; the physiology of the testes in abnormal condition; intersexuality and gynandromorphism. The report ends with a discussion of some of the "laws" of gonad activity that have been proposed. A well-selected bibliography from the world's literature closes the book. The work throughout mirrors the matured judgment and personal familiarity of the author with the subject.

The volume represents an excellent piece of book-making. The illustrations are numerous and well selected.

ABSTRACTS

Studies on physiology of reproduction; changes in adrenal gland of female rat associated with oestrous cycle. Anderson, Dorothy H. and Helen S. Kennedy, J. Physiol. **76:** 246. 1932.

A statistical study on the relation of the weight of the adrenal gland in oestrus and dioestrus to body weight, the cortex-medulla ratio, and the histological changes in the adrenal glands during these cycles was made. The results show that the absolute and relative weights of the adrenal glands of virgin female rats are greater during oestrus than in dioestrus. In dioestrus the relative adrenal weight was fairly constant, 0.20-0.23 gm. per kg. of body weight regardless of the weight of the rat or the regularity of the oestrus cycle. The mean relative weight of the adrenal gland at oestrus and dioestrus in 63 animals weighing less than 160 gm. and having regular cycles was 296 ± 27 mg./kg. at dioestrus, respectively. The mean absolute weight of the adrenal glands was greater in the small animals. The augmentation in the weight of the adrenal glands was due to an increase in the size of the cortex. A decided enlargement of the cells of the zona fascicularis and of the cells near the medulla took place during oestrus.—L. B. Nice.

Relation between the cholesterol content of the blood and adrenaline sensitivity (Untersuchungen über der Zusammenhang zwischen dem Blutcholesteringehalt und der Adrenalinempfindlichkeit). Babarczy, Marie v., Arch. f. exper. Path. u. Pharmacol. **160:** 699. 1931.

In 16 cases the sensitivity to adrenaline was measured by noting the change in blood pressure after the intravenous injection of 0.01 mg. of the drug, and the level of the blood cholesterol was also determined. While in the majority of cases a high or upper normal value for blood cholesterol was accompanied by an increased sensitivity, nevertheless no fixed relationship could be demonstrated between the two functions.—J. M. Looney.

Calcification of the suprarenal glands in Addison's disease. Camp, J. D., R. G. Ball and C. H. Greene, Am. J. Roentgenol. **28:** 594. 1932. Abst., Am. J. M. Sc. **186:** 146.

Roentgenologic evidence of calcification of the suprarenal glands in cases of Addison's disease has been presented in numerous isolated case reports, but the frequency with which such evidence can be obtained has not heretofore been determined. Accordingly the authors have tried to determine: (1) The incidence of calcification in a series of suprarenal glands removed at necropsy in cases of Addison's disease; (2) the incidence of calcification in the suprarenal areas in a consecutive series of patients affected with Addison's disease. The suprarenal glands were obtained in 34 cases which had been listed by the pathologists as Addison's disease or as tuberculosis of the suprarenal glands. A positive clinical diagnosis of Addison's disease had been made in 23 of these cases, and a questionable diagnosis in 3; in the remaining 8 cases there were tuberculous lesions of one type or another. A second group of suprarenal glands adjudged to be normal was also observed for control. Both groups were subjected to roentgenologic examination. Of the 23 cases in which a positive diagnosis of Addison's disease had been made, 6 showed roentgenologic shadows of calcification; of the 3 questionable cases, 2 showed such shadows; of the 8 cases of tuberculous glands, 3 were positive. Thus 32.3% of the entire group showed shadows of calcification. These types of calcification were noted: (1) Gross calcification of the entire gland; (2) multiple discrete areas of calcification; (3) homogeneous increased density of the entire gland, suggesting a diffuse deposition of lime salts. Twenty-three patients having Addison's disease clinically were then examined with the Roentgen rays and shadows indicative of suprarenal calcification were observed in 6.

Effect of injections of extracts of adrenal cortex on development and sex functions of albino mouse. Cleghorn, R. A., J. Physiol. **76**: 193. 1932.

An adrenal cortex extract prepared by the Swingle and Pfiffner method was injected subcutaneously daily into 18 white mice by beginning on the twenty-first day of age and continuing for a period of four weeks. Twenty-one control mice received similar amounts of an inactivated extract or a saline in the same manner. There was practically no difference between the weights of the control and experimental male mice or between the control and experimental female mice, respectively, at the end of the experiment. Neither was there any appreciable influence found in young female mice as to the time of opening of their vaginal canals nor on the oestrous cycle in adults.—L. B. Nice.

The output of epinephrine from the adrenal glands during anaphylactic shock. Cohen, M. B., J. A. Rudolph and J. M. Rogoff, Proc. Am. Physiol. Soc. P. 21. 1933.

The epinephrine output from the adrenal glands, during anaphylactic shock was studied in nine dogs. The method of Stewart and Rogoff was employed; this is the only available method permitting satisfactory quantitative measurements for determining the rate of epinephrine secretion. All the animals were sensitized with horse serum, 5 cc. on two successive days. The first dose was administered subcutaneously in others. The shock experiments were performed four to five weeks after administration of the sensitizing serum. In two animals intravenous injection of 5 cc. horse serum failed to produce shock, in all the others definite shock was obtained, varying in degree. The maximum fall in blood pressure was 90 mm. Hg., the minimum 24 mm. The maximum initial pressure observed before shock was 140 mm., the minimum 74 mm. The maximum pressure observed during shock was 50 mm., the minimum 16 mm. The other characteristic phenomena of anaphylaxis in dogs was observed in all the animals, varying in degree with the intensity of the shock. In no case was there any evidence of interference with the epinephrine secretion from the adrenals, although in some animals the shock was very profound and the blood pressure very low, while in others the anaphylactic manifestations were relatively mild. In no case did the epinephrine output from the adrenals exceed the maximum of the normal range obtained in the large series of determinations made by Stewart and Rogoff. In the majority of cases the epinephrine output was the same before and after shock. In one animal the calculated output indicated a probable increase but in view of a very slow blood flow and the fact that the apparently increased output did not exceed the maximum of the normal range, it would not be proper to interpret this result as an actual increase. Our experiments permit the conclusion that anaphylactic shock does not alter the output of epinephrine from the adrenals.—Authors' Abst.

The effect of cortico-adrenal extract injection on the oestrous cycle of the rat. Corey, E. L., Proc. Am. Physiol. Soc. P. 24. 1933.

The oestrous cycle was studied by means of the vaginal smear method of Long and Evans. About one hundred animals were used. A control period of from one to three weeks elapsed before operation or treatment of any kind. The injection of cortico-adrenal extract (made according to a modified Swingle-Pfiffner method in this laboratory) in normal rats resulted in a variation of the oestrous cycle in the direction of increased activity. The oestrous type of smear was also often observed for several days consecutively. Continued extract administration, in some cases, brought about eventually an inhibition of oestrus. Adrenalectomy resulted in complete suppression of the cycle and ultimate death of the rats with characteristic weight losses. The injection of cortico-adrenal extract in such cases restored the oestrous cycle and maintained the animal alive for an indefinite period, none of them dying during extract administration. Withdrawal of the extract resulted in cessation of the oestrous cycle for a second time. The injection of either "Theelin" or "Antuitrin-S" beginning just prior to adrenalectomy served to maintain the cycle during the life-span of the animals. Some of these rats exhibited vaginal smears characteristic of oestrus even up to the time of death. The "Theelin" used was found effective in the case of ovariectomized rats. Cortico-adrenal extract injection resulted, in about 50% of cases, in the irregular exhibition of smears of the oestrous type in ovariectomized rats. No explanation of this phenomenon is offered at the present time.—Author's Abst.

The effects of adrenal cortex extract on the reproductive system. Grollman, A. and Evelyn Howard, Proc. Am. Physiol. Soc. P. 42. 1933.

An interrelationship between the gonads and the adrenal cortex has long been suspected on anatomical and clinical grounds. The preparation of a potent and relatively pure cortical extract has now enabled us to directly test the effects of the cortical hormone on the reproductive tract. Cortical extract prepared by us was injected daily into young rats in amounts more than five times that required to maintain life in the absence of cortical tissue. In a series of 14 males the testes averaged 20% heavier in the injected animals (per cent of body weight). In a group of 22 females no effect on the age of vaginal opening was observed, and vaginal smears demonstrated that the oestrous cycle was not significantly affected.—Authors' Abst.

Further studies on the adrenal cortex. Hartman, F. A., J. E. Lockwood and K. A. Brownell, Proc. Am. Physiol. Soc. P. 46. 1933.

The histological changes in the adrenal cortex associated with vitamin deficiencies suggested experiments with cortical extracts. Extracts containing cortin were administered in two series of animals, one deficient in vitamin C and the other deficient in vitamin B complex. Cortical extract delayed the onset of scurvy in guinea pigs receiving basal ration only and in those with a half-protective dose of vitamin C in addition to the basal diet. This was indicated both by the weight curves and by the scurvy score. Vitamin C was removed from the extract by using ethyl ether. Injections of cortical extract likewise delayed the onset of symptoms due to a diet deficient in vitamin B complex. When the extract was fed in the same amount as when injected, no protection was afforded. Results, therefore, suggest that cortical extract aids in the utilization of both the vitamin B complex and vitamin C. Carr first showed that cortical extract made according to the Swingle and Pfiffner method did not support lactation in adrenalectomized rats. Likewise our routine extract containing cortin does not support lactation. However, we have obtained a substance other than cortin from the adrenal cortex which, if added to cortin, does support lactation. With this combination it has been possible to rear from 50 to 64% of the young born by adrenalectomized mothers compared with 72% in animals reared by normal stock. The preparation which supports lactation has given positive results on the crop glands of pigeons. The evidence seems to be conclusive that the adrenal cortex produces a second hormone for which we propose the name of cortilactin.—Authors' Abst.

Effect of cortical extracts on the oestrual cycle of completely suprarenalectomized rats. Martin, S. J. and R. L. Kroc, Proc. Am. Physiol. Soc. P. 71. 1933.

Daily vaginal smears and body weights were recorded on a series of young adult female albino rats. Following bilateral suprarenalectomy, only those showing unmistakable signs of glandular insufficiency and a complete cessation of the oestrual rhythm for at least two normal cycles were injected subcutaneously twice a day with extracts of whole suprarenal glands. The extracts, found to be physiologically active in the rat, were prepared from beef and horse adrenals following Swingle-Pfiffner's long and "one-half" methods. The injected females undergoing treatment for 7 to 16 days showed a return of the oestrual rhythm of 1 to 5 cycles but only after their body weights had been restored to approximately the pre-operative level. Some of these experimental rats have been maintained by injections of extracts in apparently normal physiological condition beyond the longest survival period recorded by us; when injections were stopped, death followed in 11-14 days. A few experimental females receiving injections have been successfully mated. It is concluded that the extracts prepared show no inhibitory influence upon the normal cyclic rhythm of the ovary as reported by some investigators, and contain no specific ovary-stimulating substance.—Authors' Abst.

A case of Addison's disease treated with cortical suprarenal extract. McCrie, J. G., J. M. Mears and W. G. Millar, Brit. M. J. **2**: 622. 1932.

A single case of a female, age 17, is reported. The patient was treated with a cortical suprarenal extract (eucortone), Allen and Hanbury's. The case is most valuable clinically in aiding the establishment of dosage; however, no blood chemistry findings are reported. Post-mortem examination did not

include the pituitary gland. The suprarenals were interesting because one showed atrophic changes and the other some tendency toward regeneration. —G. W. Thorn.

Chronic adrenal insufficiency: an hitherto undescribed syndrome: with case report. Packard, M. and H. F. Wechsler, Am. J. M. Sc. **186**: 66. 1933.

A case of malnutritional edema is reported which exhibited an unusual clinical syndrome and a degenerative lesion of the adrenals at autopsy. The outstanding clinical features were extreme emaciation with complete loss of body fat, asthenia, anorexia, polyneuritis, trophic ulcers and absence of pigmentation and hypotension. The similarity between the syndrome and the state of chronic adrenal insufficiency in animals produced by bilateral suprarenalectomy is pointed out. The literature on the effect of total and partial inanition and the various vitamin deficiency on the adrenal glands in both animals and man is reviewed. It is suggested that this clinical syndrome, hitherto undescribed, is one of chronic adrenal insufficiency due to the adrenal degeneration occasioned by malnutrition (a second less severe case is cited as an addendum).—Authors' Summary.

Experimental chronic adrenal insufficiency (Addison's disease) in animals. Rogoff, J. M., Proc. Am. Physiol. Soc. P. 84. 1933.

Chronic adrenal insufficiency can be produced, in cats or dogs, by subtotal ligation of adrenal blood vessels. Such animals survive an average of four to six months following the final ligation operation. Survival periods ranging from two to 14 months have been observed. Acute or subacute clinical phenomena, characteristic of adrenal insufficiency, develop at intervals and vary in character, degree and duration. Blood changes similar to those occurring in Addison's disease and in adrenalectomized animals, occur during these exacerbations. After one or more exacerbations the cortical substance of the gland may regenerate sufficiently to lead to permanent recovery, or degeneration of the cortex may proceed to the point where an acute crisis is fatal. Selective degeneration of the cortex, without damage to the medulla, is a common result, although necrosis may occur in the entire gland, in some cases. At autopsy, the adrenal glands show pathologic changes similar and in some cases identical with those seen in certain types of human Addison's disease.—Author's Abst.

Some circulatory phenomena disclosed by ergotoxine. Rosenblueth, A. and B. Cannon, Am. J. Physiol. **105**: 373. 1933.

In experiments on cats (numbers not stated) it appeared that after a moderate dose of ergotoxine a dose of adrenin, which gives an almost pure rise of blood pressure in the whole animal, produces a pure fall if the circulatory area is limited by clamping the aorta at the diaphragm. This difference in effect does not depend on the original blood-pressure level. From this fact and other evidence previously reported, the inference is drawn that the splanchnics have primarily vasoconstrictor action and that probably sympathetic vasodilators are distributed mainly to skeletal muscle. The bearing of this selective distribution on the theory of transmission of the sympathetic nerve impulses to the effectors, recently developed by Cannon and Rosenblueth, is discussed. In the spinal animal ergotoxine caused a rise of blood pressure, whereas in the myelencephalic preparation exgotoxine produced a considerable fall. The explanation is offered that excitation of a dilator center in the medulla may be produced by ergotoxine. Although most vasomotor reflexes are readily abolished by ergotoxine, some persisted after doses of ergotoxine sufficient to transform the blood pressure response to adrenin into a pure fall. The cardiac reflexes persisted, modified by the central and peripheral effects of ergotoxine on the vagi. Some of the motor effects elicited by ergotoxine in different preparations are described. The most noticeable characteristic seems to be a marked reflex hyperexcitability of all motor centers. Muscular activity was found to lower the blood pressure of ergotoxinized animals. Injection of curare, on the contrary, was followed by a rise. The central effects of ergot appear thus to be complex: (a) paralysis of the respiratory center; (b) abolition of most vasomotor reflexes without abolition of cardiac reflexes; (c) excitation of the vagal center; (d) excitation of a dilator center in the medulla; (e) increased reflex excitability of the spinal and encephalic motor centers; (f) direct excitation of motoneurones.—R. G. H.

Disappearance of vitamin C from adrenals of guinea pigs having scurvy. Siehrs, A. E. and C. O. Miller, Proc. Soc. Exper. Biol. & Med. **30**: 696. 1933.

The authors investigated the disappearance of vitamin C from the adrenals of guinea pigs on a scorbutic diet and the time for its reappearance when orange juice was added to the diet. Reduction of silver nitrate solution by the cut surfaces of the adrenals was the criterion used. They conclude that the amount of vitamin C stored in the adrenals is smaller than is generally thought and that the supply is rapidly lost in 3 to 6 days by excretion or metabolism. Considerable time elapses between the disappearance of vitamin C from the adrenals and the development of the gross symptoms of scurvy. The vitamin is rapidly returned to the adrenals when orange juice is fed.—M. O. L.

Studies on suprarenal insufficiency; the growth of transplanted cortical tissue in the rat. Wyman, L. C. and Caroline tum Suden, Am. J. Physiol. **101**: 662. 1932.

Studies were made on 143 suprarenalectomized albino rats having autoplastic transplants of cortical tissue, "accessory" cortical tissue, or both. There were 69 females and 74 males in the series. Measurements of the diameters and weights of the regenerated cortical masses showed that females regenerate more cortical tissue than do male rats. The total amount of tissue regenerated is fairly constant for each sex, irrespective of the number of masses present. Apparently in the rat there is a limiting factor, differing in the sexes, which regulates the amount of cortical tissue which can regenerate from transplants, from fragments left at operation, or from cortical rests. Obviously the transplantation method cannot be used to induce hyperfunction of the suprarenal cortex. Homoplastic transplants of cortical tissue in rats are successful, and will grow in the presence of autoplastic transplants made at the same time. Heteroplastic transplants are unsuccessful. Neither autoplastic nor homoplastic transplants of cortical tissue will grow in the presence of both, one, or even a good sized fragment of one of the animal's own suprarenal glands. This is further evidence for a physiological factor limiting the growth of suprarenal cortical tissue.—Authors' Abst.

Constitution and constitutional anomalies. Authors, numerous, Endokrinologie, **12**: 356. 1933.

A notable collection of abstracts of recent articles.—R. G. H.

Genetics, especially human. Authors, several, Endokrinologie, **12**: 371. 1933. Abstracts recent literature.—R. G. H.

Antitrypsin; the hormonal and pharmacological relationship between the albumin-globulin ratio and the antitryptic titer of human serum (Über hormonal und pharmakologishe Beeinflussung des Albumin-Globulinquotientn und des antitryptischen Titers in Serum des Menschen). Chrometzka, F., Ztschr. f. d. ges. exper. Med. **80**: 408. 1932.

The author investigated the effect of parenterally introduced endocrine products and vagotonic and sympatheticotonic substances on the antitryptic titer and the albumin to globulin ratio. Fifteen cc. of blood were taken before and after the injections. The author used thyroxine, hypophysis extract, and "präphyson" in a single case each, and insulin, adrenaline, atropin, and pilocarpine in two cases. Thyroxine and präphyson increased the antitryptic titer, while insulin and hypophysis extract decreased it. The effect of adrenaline, atropin, and pilocarpine was dependent on the albumin-globulin ratio. If this was high, the injection caused a decrease in the antitryptic titer, while if it was low there was an increase. The albumin-globulin ratio is lowered by thyroxine and präphyson, and raised by insulin and hypophysis. It is concluded that the antitryptic titer is regulated neurohormonally through the albumin-globulin ratio, which in turn is affected by certain vagotonic and sympatheticotonic substances.—J. M. Looney.

The therapy of hemorrhages in the sexually mature woman (Die Therapie der Blutungen bei der geschlechtsreigen Frau). Halban, J., Wien. med. Wchnschr. **82:** 14. 1932.

Treatment of uterine bleeding presents a difficult problem owing to the variety of conditions causing it and associated with it. A general discussion of the problem emphasizes the importance of accurate diagnosis of the kind and cause of bleeding as a sound basis for therapy. Symptomatic treatment is deplored and causal therapy recommended. In the diagnosis, distinction between physiological and pathological bleeding is urged. In pathological bleeding functional is to be distinguished from organic; the menorrhagias from the metrorrhagias. Functional bleeding is attributed to disturbed function of the ovary. Many organic causes are mentioned: viz., all causes which produce hyperemia of the ovary, inflammations, systemic, cardiac failure, asthenia, anemia, psychic, obesity, tumors and misplacements. In the functional bleeding the ovary is to be considered a link in the chain of endocrine glands, therefore each and all of these glands are to be surveyed. The hypophysis is considered the controlling organ of the ovary. Therapy should always be causal. Among the gland products mentioned are those from the hypophysis, both anterior and posterior lobe; thyroid, insulin, ovarian. The general therapeutic measures include correction of disturbed metabolism (obesity and undernutrition) and anemias. To increase the coagulability of the blood injections of horse serum, defibrinated blood, x-ray irradiation of the spleen and liver are procedures not often effective; transfusion of blood is often helpful. Baths are useful, especially in inflammations. Among the local measures considered favorable are baths and hot and cold vaginal douches. Radium may be used but is not favored. Roentgen ray castration is ideal during or near the climacteric, but is contraindicated in the young. If there is any suspicion of carcinoma, hysterectomy, preferably vaginal, is indicated. Curettage alone is occasionally sufficient. Partial resection of the ovaries is especially favored. Personal qualifications of the operator are important in determining the employment of operative procedures. No one method of treatment is applicable in all cases. An accurate diagnosis should precede causal therapy. The author has given a sound conservative general exposition of the subject, omitting details.—J. P. P.

Small doses of x-ray for amenorrhea and sterility. Edeiken, L., Am. J. Obst. & Gynec. **25:** 511. 1933.

The author treated 56 patients showing functional amenorrhoea with small doses of x-rays directed to the pituitary body and the ovaries, and obtained favorable results in 40. Two of the patients showing favorable results received irradiation of the pituitary body alone. He believes that this is added clinical proof of the relationship of the hypophysis to ovarian function. There was an associated functional sterility in 33 cases. The following results were obtained in this series: Sterility patients, 33; total pregnancies, 15 (14 patients); delivered normal children, 11; premature delivery of normal infant at 7½ months. (Pneumonia developed and death occurred in six days), 1; pregnant but not delivered, 3. The author concludes: (1) Small doses of x-rays directed to the pituitary gland and ovaries are of proved value in patients with functional amenorrhea. (2) It is also of definite value in properly selected cases of female sterility. (3) Before irradiation is undertaken all pathologic lesions in the pelvis, which may be a possible cause of amenorrhea or sterility, must be excluded by a competent gynecologic examination. (4) This form of treatment is not attended by any reaction or discomfort to the patient, and when properly administered has no ill effects upon the patient or the subsequent offspring. (5) To obtain the most satisfactory results, coöperation is essential between the gynecologist and the radiologist.—Author's Abst.

Biological characteristics of ovary-stimulating extracts made from blood of pregnant women. Fluhmann, C. F., Proc. Soc. Exp. Biol. & Med. **29:** 1193. 1932.

A method of preparing an estrin-free ovary-stimulating extract from blood of pregnant women is described. This extract was found to induce a luteinization of the ovaries of immature rats as well as a marked increase in uterine weight. As compared to anterior pituitary implants it produced comparatively little increase in ovarian weight in five-day experiments.—Author's Abst.

On the etiology of nymphomania of the cow (Zur Ätiologie der Nymphomanie der Kuh). Frei, W., Arch. f. Gynäk. **147**: 836. 1931.

Nymphomania in the cow is described and discussed in the light of recently acquired knowledge regarding the hormonal control of the reproductive organs. Persistent follicles or follicular cysts are regarded as the most common causative agents and the inter-relationship between ovary and hypophysis is consequently considered. It is stated that persistent follicles may resemble the normal mature follicle as regards size and histology and thus means of identifying the persistent follicle are not made entirely clear. The relation of nutrition to the functioning of the ovary is discussed. Abnormalities of the oviduct, uterus or vagina may also result in the expression of symptoms of nymphomania.—H. H. Cole.

On the hormone therapy of oligo-hormonal secondary amenorrhea (Zur Hormontherapie bei oligohormonaler, sekundärer Amenorrhöe). Gragert, O., Zentralbl. f. Gynäk. **56**: 2463. 1932.

The author advocates the combined administration of sex hormone (estrin) and anterior pituitary hormone (prolan) for the treatment of secondary amenorrhea. Five cases of amenorrhea of from 3 to 16 months' duration in young women are described in detail.—C. F. Fluhmann.

Some effects of restricted protein intake on the oestrous cycle and gestation in the rat. Guilbert, H. R. and H. Goss, J. Nutrition, **5**: 251. 1932.

Sexually mature rats varying in age from 50 to 100 days were fed diets varying in protein content. Diets containing from 3.5 to 5.0% protein resulted either in cessation of oestrus or in long and irregular cycles. Some of the rats on a diet containing 5% protein which continued to exhibit oestrus were subjected to breeding tests. No litters were produced. The lack of fertility was of three general types: (a) Failure to mate when placed with normal males during oestrus; (b) apparently infertile matings with recurrence of oestrus, the condition of pseudopregnancy not being induced; (c) fertile matings followed by the death of the embryos, the incidence of which was denoted by the appearance of blood in the vagina on the 8th to the 12th day following coitus. Diets containing 7.5 to 8.0% protein permitted growth at only slightly less than the normal rate and the oestrous cycle was normal. Histological examination of the ovaries revealed that numerous corpora remained as long as 70 days after the last period of oestrus. There were also numerous follicles, many of which, both large and small, were undergoing atresia. There were few primordial follicles and there was an abnormal amount of interstitial tissue.

—P. B. Pearson.

An international standard for the estrus-producing hormone. London correspondent, J. A. M. A. **101**: 377. 1933.

In July, 1932, a conference was held in London under the auspices of the Permanent Commission on Biological Standardization of the Health Organization of the League of Nations, to discuss international agreement on standards of reference and units of activity for the sex hormones. It was agreed that knowledge of the male sex hormone and of the hormones of the corpus luteum and anterior pituitary body is sufficiently advanced to permit a standard of reference being established and a unit of activity defined. Important decisions were also reached as to the estrus-producing hormone, which has been prepared from the urine of pregnancy in quantities sufficient for chemical examination. It exists in two forms—a ketohydroxy and a trihydroxy. The former is the more active in producing estrus in animals from which the ovaries have been removed. The conference agreed on the adoption of a standard substance in terms of which the unit could be defined as the only safe basis for international use. The ketohydroxy form of the hormone in pure crystalline condition, preserved at the National Institute for Medical Research, London, was adopted. The unit of activity was defined as that contained in 0.0001 mg. of this substance. In order to prove an adequate amount of material to serve as an international standard, different countries have sent samples of the pure crystalline ketohydroxy form of the estrus-producing hormone to the National Institute of Medical Research, London, which for this purpose is acting as the central laboratory on behalf of the Health Organization of the League of Nations. At the institute the final preparation of the standard has been com-

pleted and arrangements have been made for its storage and dispatch to the laboratories in other countries, which have been nominated by the Health Organization of the League of Nations.

The chemistry of oestrin; mechanism of conversion of trihydroxyoestrin into ketohydroxyoestrin. Marrian, G. F. and G. A. D. Haslewood, Biochem. J. **26**: 25. 1932.

The authors show that the acidity of ketohydroxyoestrin is not due to enolization of the carbonyl group but is due to acidic ionization of the phenolic hydroxyl as is true in the case of trihydroxyoestrin. This is proven by the fact that dehydration of the monomethyl ether of trihydroxyoestrin yields the methyl ether of ketohydroxyoestrin. Neither ether derivative has acidic properties. Therefore, the carbonyl group must be formed by the removal of water from the two non-acidic hydroxyl groups. The physical constants of a number of derivatives are given.—J. M. Curtis.

The origin of the comb-growth-promoting substance in male urine. McCullagh, D. R., W. K. Cuyler and J. T. Frawley, Tr. Roy. Soc. Canad. **26**: 183. 1932.

The physiological and physico-chemical properties of the substance from urine which promotes comb-growth in capons have been studied and compared with the properties of testicular extracts. Preparations from urine produced changes in the seminal vesicles and prostates of rats which could not be distinguished from changes produced by the testicular preparations. The substances from different sources could not be distinguished by the spermatozoon motility test or the ejaculation test. The solubility and thermostability of preparations from testes and urine were the same. The active principle was not found in the urine of eunuchs. It is concluded that the substance in the urine of males, which produces comb growth when injected into capons, is of testicular origin and is probably identical with the testicular hormone.
—D. R. McCullagh.

Further observations on testicular extract and its effect upon tissue permeability. McLean, D., J. Path. & Bact. **34**: 459. 1931.

The author confirms and extends former experiments concerning the increase in tissue permeability following injection of testicular extracts. The active principle is soluble in water, insoluble in alcohol and ether and can be sterilized by dry heat at 160° C. It is tested by intradermal injection "in vivo" and "in vitro." The rate of diffusion of the inoculum gives a measure of the activity of the preparation. Histological examination demonstrates swelling and splitting of the fibre bundles of the skin. The changes in permeability are not related to surface tension. Extracts of spermatozoa possess the same activity as extracts of whole gland. The permeability of ova appears to be increased by these extracts. The preparations are inactivated by tryptic digestion. The active principle has not been isolated. Clupeine sulphate, a characteristic prolamine salt, gives the same changes in permeability.—D. R. McCullagh

Experimental studies on the problem of sex inversion; a contribution on the question of antihormonal sterilization of male animals (Experimentelle Studien zum Problem der Geschlechtsumwandlung; ein Beitrage zur Frage der antihormonalen Sterilizierung männlicher Tiere). Neumann, H. O., Ztschr. f. Gerburtsh. u. Gynäk. **101**: 568. 1932.

A large number of experiments are reported on the effect of ovarian transplants and female sex hormone preparations upon the reproductive organs of male mice, guinea pigs and rabbits. Both ovarian transplants and female sex hormone caused mammilary development in the male. The female sex hormone was found to have an injurious effect upon the testes leading to a temporary sterilization in some animals.—H. H. Cole.

Factors influencing the distribution and character of adipose tissue in the rat; effect of ovariectomy and of feeding with thyroxine. Reed, L. L., W. E. Anderson and L. B. Mendel, J. Biol. Chem. **96**: 313. 1932.

Ovariectomy of young rats resulted in the deposition of a smaller proportion of adipose tissue in the genital fatty depots and of a correspondingly larger proportion under the skin than was exhibited by controls. The iodine

numbers of the fats were similar. When groups of rats were fed 0.5 to 2 mg. of crystalline thyroxine daily for 10 and 8 weeks, respectively, the distribution of the stored fat was not altered, whereas the iodine number of the fat was raised by 10-15 units.—L. R. Farquhar.

Uterine bleeding of monkeys in relation to neural and vascular processes; spinal transection and menstruation. van Wagenen, G., Am. J. Physiol. **105**: 473. 1933.

The spinal cords of seven normal monkeys (two mangabeys, Cercocebus fuliginous, and five Rhesus monkeys, Macaca mulatta) were transected at different times in the earlier half of the menstrual cycle. This procedure was followed in all cases by bleeding from the uterus after an interval of from two to nine days. Transection was performed at different levels between the eighth and the twelfth thoracic segment. The latent interval preceding the induced bleeding appears to be shorter the lower the cord is divided. The operative cycle, which is regarded as comprising the number of days from the first day of the last pre-operative bleeding to the beginning of the first post-operative bleeding is considerably shorter than the mean length of the menstrual cycle in unoperated animals. Following the post-operative bleeding, the normal menstrual rhythm is reëstablished. Both the uterus and the ovaries undergo atrophic changes after the operation. Experiment showed that the sexual skin remains sensitive to theelin. Transection of the spinal cord in a single castrate animal was not followed by uterine bleeding.—Author's Summary.

The experimental study of senescence. Wiesner, B. P., Brit. M. J. **2**: 585. 1932.

Studies were made on a highly inbred stock of rats, bred from one ancestral pair of Wistar rats, the colony being maintained at a strength of about 3000 animals. Environmental conditions were kept as constant as possible. Early growth was rapid and early mortality low, but after the eighteenth month of life emaciation was frequent and the duration of life, both average and maximum, appeared to be rather short. Adequate vital statistics cannot be furnished at present, but the oldest age observed in the male was 1004 days, in the female 982 days. Two types of aging involving presence or absence of manifest senescence, respectively, were observed. About half of the aged animals appeared in good condition with respect to fur, reflexes, etc., and died quite suddenly. No consistent cause of death was found and the weight curve remained "plateaued" until shortly before death. In the other animals senescence was manifest, i.e., sluggishness, reduced activity, irregular respiration, pale eyes, thin fur, and a downward trend of the weight curve. Senescence seemed to become manifest more readily in the female than in the male. Most aged males which did not exhibit manifest senescence continued to show spermatogenesis and functional seminal vesicles. In several cases, however, the testicles had ceased to produce sperm and were reduced in size. Similar conditions were found in aged males showing manifest senescence. Observations on ovarian function in aged females showed conditions resembling those found in the male, i.e., no correlation of senescence with absence of ovarian function. Apparently senescence is not absolutely correlated with absence of ovarian or testicular function, and can develop in males in which excessive testicular secretion may be assumed to exist. Conversely, aged animals may preserve a satisfactory general condition, although gonadic function is reduced. In a number of aged males and females in which ovary and testicle were found to show senile changes and reduced endocrine function, one gonad was removed for study. In six animals examined later no compensatory hypertrophy had occurred. Fourteen of the animals were treated with gonadotropic hormones (prolans) prepared from pregnancy urine, and in three cases anterior lobe grafts were given. Within 11 to 21 days restitution of gonadic function occurred in the females. In the males the findings varied. Enlargement of the testicle was found in six cases. All animals showed enlargement of the seminal vesicles, spermatogenesis was restored in four, and in the others the changes were slight. No marked effect on the general condition of the rats was seen. Apparently the senile gonad can be reactivated in some cases. Senescence of the gonad seems to be due to the lack of the gonadotropic hormones of the anterior lobe, and not to any general condition of the organism, or to the absolute age of the gonad itself. Experiments with the injection of growth-promoting extracts from the anterior lobe showed that many senile animals can react with considerable growth. In some cases the administration of

growth extracts appeared to have a beneficial influence upon the general condition of the rat. These experiments, however, are preliminary and definite conclusions cannot be drawn at present.—L. C. W.

Embryonal carcinoma of abdominal testis in a pseudohermaphrodite. Vastola, A. P., J. A. M. A. **101**: 111. 1933. Abst., A. M. A.

The author reports the case of a male pseudohermaphrodite, aged 33, with retained abdominal testes, who developed the symptoms of an acute condition of the abdomen associated with severe shock. Physical examination showed rigidity of the rectus muscles and a tumor mass rising from the pelvis nearly to the umbilicus. There were no clinical evidences of metastases. Exploratory laparotomy revealed torsion of the pedicle of an embryonal testicular carcinoma which had ruptured and bled in the abdominal cavity. The patient recovered from the operation and had a negative Aschheim-Zondek reaction over a period of six months. This, together with the fact that he had no symptoms of recurrence or metastases, pointed to clinical cure of his condition. The author states that the Aschheim-Zondek reaction is a reliable test for differentiating between malignant and benign neoplasms of the testes. It is also valuable in detecting postoperative recurrences or metastases and observing the results of roentgen therapy in such cases.

A case of basophil adenoma of the anterior lobe of the pituitary: "Cushing's syndrome". Bishop, P. M. F. and H. G. Close, Guy's Hosp. Rep. **82**: 143. 1932.

The authors report a case of pituitary basophilism (Cushing's Syndrome) in a 22-year-old female, who stopped growing and became obese at the age of 11; menstruation began at 14 and she became amenorrhoeic one year later except for three consecutive months at the age of 18; severe monthly headaches were experienced at the age of 14. Striking features at 22 were ruddy complexion; dry, scaly skin; hirsutism; stunted growth (height 4 ft. 3 in.). Beard growth required use of a safety razor. Deformity of left wrist and right hand due to fissured fracture of lower end of radius and rarefaction of the head of fourth right metacarpal bone was noted. Polydipsia and troublesome headaches were complained of. Hypertension reached as high as 300 mm. Hg. She had convulsions while in the hospital. Her fasting blood sugar was high (.14 gm. per 100 cc.). There were albumin and sugar in the urine. She died with symptoms of lung edema. Important postmortem findings were: Subcutaneous hemorrhages, edema of lungs, hypertrophied heart, extensive arteriosclerosis throughout the vessels, normal suprarenals, small kidneys, infantile uterus and small ovaries without visible evidence of Graafian follicles but present microscopically. Having read Cushing's paper on basophilism, the authors studied the pituitary, which fortunately had been preserved and found a definite basophil adenoma near the front of the anterior lobe.—R. C. Moehlig.

The effect of Loeb's anterior pituitary extract upon the basal metabolism of dogs. Bueno, J. G. and B. O. Barnes, Proc. Am. Physiol. Soc. P. 15. 1933.

Six normal and one completely thyroidectomized dogs were injected daily for 7 days with 10 cc. of Loeb's anterior pituitary extract. In the normal animals a rise in basal metabolism occurred which reached a peak of about 30% above normal on the fourth or fifth day. Other changes observed include increased respiration, increased heart rate, thirst, polyuria, and a slight rise in body temperature of two dogs. All the symptoms disappeared about five days after the last injection. None of the above changes were noted in the thyroidectomized animal. These experiments are in harmony with the view of Loeb and others that a substance is present in the anterior pituitary of cattle which stimulates the thyroid gland.—Authors' Abst.

Xanthomatosis and the central nervous system. Davison, C., Arch. Neurol. & Psychiat. **30**: 75. 1933.

A case of xanthomatosis—the forty-ninth in the literature—presenting diabetes insipidus, defects in the membranous bones and changes in the nervous system is described. In addition to the deposits of lipoid cells found in most of the organs, the white matter of the central nervous system was the seat of numerous demyelinated plaques filled with compound granular corpuscles and

giant glia cells. These two types of cells found in the neutral structures are considered analogous to the foam cells and the reactive type of cells (fibrosis and inflammatory cells) demonstrated in other organs. In this disease the hypophysis was the seat of deposits of foam cells, while the tuber cinereum region showed evidences of reactive phenomena (gliosis, fibrosis and inflammatory cells) and occasional compound granular corpuscles and giant glia cells. The process is one of disturbance in lipoid metabolism (cholesterol) and in some respects resembles Gaucher's and Niemann-Pick's diseases, and possibly amaurotic family idiocy.—R. G. H.

Carbohydrate metabolism and specific dynamic action in partially hypophysectomized dogs. Eaton, A. G. and R. G. Daggs, Proc. Am. Physiol. Soc. P. 29. 1933.

A study of glucose tolerance was made by intravenous injections at a constant rate. Partially hypophysectomized dogs showed a decreased tolerance. This was evidenced by the fact that the blood sugar rose higher than in the normal and did not return to the basal level for approximately one hour, as compared with 25 minues in the normal dog. Upon injection of one-half unit of insulin per kilo, the blood sugar reached a minimum value in 2½ hours in both normal and partially hypophysectomized dogs, but the response was much more prolonged in the experimental animals. The basal metabolism was lowered but this may be partially due to cage life. The specific dynamic action of protein was normal when computed as the average caloric increase over basal.
—Authors' Abst.

Experiments on hypophysectomized rabbits. Firor, W. M. and S. R. M. Reynolds, Proc. Am. Physiol. Soc. P. 34. 1933.

All the rabbits employed in these experiments were hypophysectomized by an intra-orbital approach, and at autopsy the completeness of the removal of all glandular tissue was confirmed by serial sections of the contents of the sellae. Urine of pregnancy was injected intravenously into three rabbits that had failed to ovulate following hypophysectomy which was completed within 35 minues post coitum. The urine of pregnancy injections induced ovulation. A second series of experiments comprise a study of uterine motility following injections of theelin into hypophysectomized pregnant rabbits. The results show that in rabbits hypophysectomized on the 5th and 6th days of pregnancy or pseudo-pregnancy the corpora lutea lose within 48 hours after operation the power to inhibit typical uterine contractions induced by theelin. The third series of experiments describe the histological alterations in the uterus and ovaries resulting from daily injections of theelin followed by daily injections of corporin (Hisaw) into hypophysectomized rabbits. The final group of experiments record the changes found in ovaries of hypophysectomized rabbits after injections of anterior lobe extract prepared by the method of Bugbee, et al.
—Authors' Abst.

Some effects of anterior pituitary extracts on nitrogen metabolism, water balance and energy metabolism. Gaebler, O. H., J. Exper. Med. **57**: 349. 1933.

Extracts of anterior pituitary containing the growth hormone were administered for 1 or 2 days to 3 adult female dogs. There was invariably a decrease in the nitrogen of the urine (all attributable to urea) and in the non-protein nitrogen of the blood, and an increased nitrogen retention. The nitrogen gained was usually soon lost again. The water intake and urine volume were greatly increased. There were marked increase in heat production and in the amount of fat oxidized. In one animal lactation occurred after some injections.
—M. O. L.

On the biology of the mammary glands. A contribution to the question of the hormone of the anterior lobe of the pituitary (Zur Biologie der Brustdrüse. Gleichzeitig ein Beitrag zur Frage der Hypophysenvorderlappenhormone). Heim, K., Monatschr. f. Geburtsch. u. Gynäk. **90**: 172. 1932.

Human colostrum, "witch's milk" from new-born infants and some other body fluids were found by Zondek-Aschheim tests on immature mice and rabbits to contain prolan A and B (called "anterior lobe hormones"). The concentration of prolan in the witch's milk increases to the fifth day after birth and this is considered to be an explanation of the fact that the actual secretion of such milk more frequently begins at the fifth to eighth day. A series of 199

cases of breast swelling with secretion in infants is reported. It is concluded that the functional development of the mammary glands is under the control of this hormone (prolan). Much literature is cited, but the recent studies of Grüter and Stricker, Corner and others dealing with the relation of ovarian hormones to mammary development, and with a true anterior pituitary hormone as the effective stimulus to milk secretion, are not mentioned.—O. Riddle.

The influence of hormones and drugs affecting the autonomic nervous system on intestinal absorption and permeability. Gellhorn, E. and D. W. Northup, Proc. Am. Physiol. Soc. P. 36. 1933.

In pithed frgs the blood vessels supplying the gut are perfused with Ringer's and Ringer's plus hormone solution, respectively, whereas the gut is perfused with isotonic glucose. Any vascular change which may occur in these experiments is compensated by appropriate changes in the perfusion pressure. Variations in the sugar concentration in the blood vessels are under these conditions the expression of a change in permeability. It is shown that acetyl choline is the antagonist of adrenaline also in regard to permeability. Acetyl choline decreases permeability in concentrations of 1:20,000,000 and increases it in higher concentrations. Extracts of anterior and posterior hypophysis are without effect on permeability, although posterior hypophysis extracts cause vasoconstriction. The effect of hormones on permeability is independent of changes in the diameter of arterioles and capillaries which they may produce. —Authors' Abst.

Hypopituitary type of obesity treated without glandular medication. Kenyon, Josephine H., J. A. M. A. **101**: 97. 1933. Abst., A. M. A.

The author treated 50 overweight patients by putting them on a modification of the high protein diet as described by Evans and Strang. She undertook this study for the purpose of determining whether a typical hypopituitary type of obesity could be successfully treated by a carefully worked out high protein diet. The diet was regarded as a treatment for obesity only and not intended to supplant endocrine therapy when indicated for other symptoms. The high protein, high vitamin diet is not advised for use by the layman but should be strictly supervised by physicians for those patients who, on careful examination, are found free from any acute or chronic diseases. The 50 patients treated showed a steady reduction to their estimated or optimal weight; the fat pads disappeared; the skin contracted, leaving no flabby folds; they held their weight loss on an unrestricted diet for periods varying from five months to more than one year; their general health improved; their blood counts and urinalyses remained normal; headaches were lessened and menstrual disturbances cleared up. There were no untoward symptoms. Other patients not included in this series are at present on the same supervised diet and show the expected steady rate of loss in weight.

The effect of anterior pituitary growth hormone on the composition of growth, and on ash, nitrogen and energy balances in rats under a paired-feeding regimen. Lee, M. O. and N. K. Schaffer, Proc. Am. Physiol. Soc. P. 66. 1933.

The composition of the gains in weight and the ash, nitrogen and energy balances are compared in control rats and litter mates of the same sex and initial weight and length given daily physiologic doses of growth hormone. The paired rats were killed and analyzed at the end of 8, 9, 10 and 11 weeks. Litter mate check rats of the same initial weight were analyzed at the beginning of the experiment. The food intake was kept the same for control and treated rats, absolutely and not relative to body weight or to surface area, despite the fact that the total weight of the 12 treated rats at the end of the experiment exceeded that of the 12 controls by 507 grams, or the equivalent of two adult rats. The excess gain of the treated rats over the controls in body length was 14.9 cm. and in body surface was 429 sq. cm. The composition of the 764 grams gain of the controls was: water, 45.2%; fat, 39.3%; ash, 3.16%; nitrogen, 2.15% and energy 4.53 calories per gram. The composition of the 1295 grams gain of the treated rats was: water, 63.3%; fat, 13.3%; ash, 3.71%; nitrogen, 3.12%; and energy, 2.32 calories per gram. The constituent gains in terms of the original composition were: for the controls—water, 22.8%; fat, 96.8%; ash, 23.1%; nitrogen, 20.7%, and energy, 60.9%; for the injected —water, 54.9%; fat, 56.3%; ash, 46.4%; nitrogen, 51.5%, and energy, 53.5%. The composition of the gain in the treated animals thus differed markedly from

that in the controls but was closely similar to the initial composition of the animals. The total nitrogen and ash eliminations of the controls (in urine, feces and shed hair) exceeded those of the treated rats by approximately the same amounts as the relative retentions by the treated rats, as determined by tissue analysis. The relative energy retention was slightly in favor of the controls.—Authors' Abst.

A rare case of polydactylism (Pripad vzácné polydaktylie s dystrofií adiposo-genitální). Mikula, M., Bratisl. lekár listy. **13:** 208. 1933.

The case is reported of a boy 3½ years old who showed the unusual number of 14 fingers and 12 toes, all well developed. He was extremely fat, had undeveloped external genital organs, showed strabismus and probably a contraction of the vision-field. Polydactylism together with adiposogenital dystrophy is a syndrome of Bardett-Biedl and in the present case only retinitis pigmentosa and the tower-like form of the head were missing to make that clinical picture a complete one.—Author's Abst.

The effect of follicular hormone on the untimely secretion of milk (Die Wirkung des Follikelhormons auf unzeitige Milchabsonderung). Offergeld, H., Therap. d. Gegenw. **73:** 113. 1932.

A multiparous woman of 48 years lactated during three previous years particularly at those periods when menstruation should but did not occur. The ovaries were small; retroversion of the uterus and some inversion of the vaginal wall were found. A fairly large fibroma could be palpated in each mammary gland. Treatment sought to restore regular cycles. This was initially tried with "hypophysis" and theelin (progynon) which after a few days led to slight bleeding and cessation of milk secretion. After 15 days another injection of theelin (450 m.u.) was followed (2 days) by copious bleeding. Thereafter the fibroma decreased somewhat in size and sensitivity, menstruations were normal despite the patient's advanced age, and lactation did not recur. The follicular hormone, arising under pituitary control, is considered of much importance in all aspects of genital function.—O. Riddle.

The preparation, identification and assay of prolactin—a hormone of the anterior pituitary. Riddle, O., R. W. Bates and S. W. Dykshorn, Am. J. Physiol. **105:** 191. 1933.

A new anterior pituitary hormone, called prolactin, was shown to be neither prolan nor the growth nor the gonad (and thyroid) stimulating hormone of the anterior hypophysis. It caused (a) secretion of milk from the prepared mammary glands of normal and castrated guinea pigs of both sexes (25), of normal and castrate female rabbits (15), of hypophysectomized rats (2), and of normal and castrated female monkeys (3); (b) growth with secretion in the crop-glands of doves and pigeons of both sexes (400), including one without hypophysis and 5 castrates. Prolactin was prepared by alkaline (also acid) extraction of bovine or sheep anterior lobes, removal of an inert precipitate at pH 7.5-8.0, followed by precipitation and washing of the prolactin fraction at pH 5.0. Prolactin so prepared gives no stimulation of the immature bird testis. Various treatments cited by others as destroying the growth hormone did not destroy prolactin. The fraction soluble at pH 5.0 contained the gonad-stimulating (sex-maturity) hormone. When either this latter fraction, the "aqueous pyridine and water-soluble" fraction of Fevold, et al., the thyreotropic fraction of Collip, or commercial antuitrin was injected, the testes of immature doves were enlarged 3 to 10 times within 4 to 10 days with little or no enlargement of the crop-glands. Prolan (antuitrin-S, and fresh preparations) had no effect upon either gonads or crop-glands of doves or pigeons. Growth hormone preparations of Lee and Schaffer, Collip, and Van Dyke gave only traces of crop-gland stimulation and very rarely a lactation response. An Evans' growth preparation and antuitrin-G strongly stimulated the crop-gland. All growth preparations, except that of Collip, Selye and Thomson, strongly stimulated the testis of the immature dove. Numerous tests proved that pure gonad-stimulating and growth hormone stimulated neither the crop-gland nor lactation; and other tests demonstrated that these two responses are induced by the same hormone—prolactin. Prolactin induced secretion of milk beginning about the fourth day in guinea pigs and after 2 to 3 days in rabbits. Daily injections of prolactin into doves or pigeons increased the weight of the

crop-glands up to 7 days, when the maximum was attained. A method for the quantitative assay of prolactin was developed, using the crop-gland response of doves and pigeons, injecting once daily on four successive days, killing on the fifth day (96 hours) and weighing the two crop-glands. When the crop-gland weights of doves and pigeons, corrected to the same body weight, were plotted against the logarithm of the quantity of prolactin injected, the points were found to fall about a straight line of characteristic slope. Such a line was proposed as a standard for determination of potency. Mature birds were found to respond to one-fourth of the amount of prolactin required in the immature, but the line relating dose and response was found to have the same characteristic slope as that found for immature birds. Prolactin at pH 7.5-8.5 heated for one hour at 100° showed little loss of potency. Sodium chloride increased the rate of destruction.—R. W. Bates.

Thyroid hypertrophy as a response to the gonad-stimulating hormone of the pituitary. Riddle, O., R. W. Bates and S. W. Dykshorn, Proc. Soc. Exper. Biol. & Med. **30**: 794. 1933.

It is shown that 2 anterior pituitary hormones—prolactin and the growth principle—do not cause the thyroid hypertrophy which characteristically follows the injection of various pituitary extracts. Hyperplasia of the normally developed thyroid following pituitary administration is a specific response to the gonad-stimulating hormone, or to another pituitary derivative having very similar solubilities. Good gonad-stimulating preparations do not invariably induce an increase in thyroid weight in doves and pigeons.—M. O. L.

The effect of castration on the hypophysis cerebri of the male albino rat. Stein, S. I., Anat. Rec. **56**: 15. 1933.

In agreement with other authors, it was found that the average weight (8.0 mg.) of the whole hypophysis in a group of 27 castrated male albino rats was significantly greater than the average weight of the whole hypophysis (12.0 mg.) in a group of 16 control animals. By means of the paper weight method on serial sections, it was found that this increase in size is due solely to an increase in size of the pars anterior. Pars posterior and pars intermedia are practically identical in absolute weight in the control and test animals. A difference of 20 days in the duration of castration did not materially change the picture. Removing the epididymis with the testis in the operative procedure gave the same results as leaving the epididymis intact. An accumulation of colloid varying from 0.2% to 5.0% of the total weight of the gland was found in the test glands. Practically none was seen in the control glands. A significant decrease in body weight and body length was noted in the test animals. Preliminary inspection of the sections shows many large basophilic castration cells, a great number of eosinophils, and a relatively small number of neutrophils.—Author's Abst.

Effects of pituitary antidiuresis on non-cardiac edema. Thompson, W. H., Mildred Ziegler and I. McQuarrie, Proc. Soc. Exper. Biol. & Med. **30**: 16. 1932.

The induction of pituitary antidiuresis in non-edematous patients is followed by a marked net loss in body weight due to a subsequent diuresis. This finding suggested a study of its effect on the water and mineral exchange in the presence of edema. The cases studied were: Two typical lipoid nephroses, a nephrotic glomerulonephritis and one edema of undetermined etiology. The total serum proteins were low in all cases. Complete mineral and water balances under ideal metabolic conditions were made; first during a control period, second during subcutaneous administration of antidiuretic pituitary extract (pitressin P.D.) until body weight had increased about 5%, and third during recovery period. The results were: (1) Forced water retention during pitressin period caused visible increase in edema in all subjects; (2) that the temporary water storage occurred in the extracellular tissue spaces was indicated by the fact that the chief minerals finally excreted with it were Na and Cl; (3) the response was greatest in pure nephrosis, least in chronic glomerulonephritis and intermediate in the edema of undetermined etiology; (4) as in the normal subject, strongly negative NaCl balances occurred during and immediately after the antidiuresis in the pure nephrosis; (5) the net loss of body water is roughly proportional to the excess Na and Cl excreted, in one case almost a complete loss of edema; (6) except for slight flushing out of K during diuresis, no significant changes in the other mineral balances occurred.—W. H. Thompson.

Cyclic histological variations in the anterior hypophysis of the albino rat. Wolfe, J. M. and R. Cleveland, Anat. Record, **55**: 233. 1933.

There are cyclic histological changes in the anterior hypophysis of the rat which may be correlated with the different phases of the oestral cycle. These changes are qualitative, rather than quantitative. During oestrus the cells of type I (eosinophiles) are well packed with granules which stain rather intensely with orange G. During metoestrus and dioestrus the granules in the cells are less closely packed and take a lighter stain. During late dioestrus and proestrus the cells of type III (basophiles) are filled with fine granules which stain deep blue. During oestrus and metoestrus most of these cells contain no granules and appear as large, round cells with a light blue cytoplasm. These results are based on a study of anterior lobe material from 40 rats. Complete serial sections were cut of all glands. One section from each ribbon of 25 was mounted, thus providing a complete set of interrupted serial sections of all glands. Cell counts were made on every fourth section of each set. A total of 178 sections were studied and 119,396 cells counted. There are no definite variations in the relative percentages of the various cell types present at the different phases of the oestral cycle; the qualitative changes mentioned above are definite, however.—J. M. Wolfe.

Insulin resistance due to allergy. Allan, F. M. and I. R. Scherer, Am. J. M. Sc. **185**: 815. 1933.

Allergic reactions to insulin have become less frequent but still occur in a significant number of cases. Serious symptoms are uncommon, and alteration of the action of insulin has seldom been encountered. A report is given of a subject with diabetes who became refractory to insulin because of allergy. Coma developed and at first could not be overcome with massive doses of commercial insulin. Treatment with solution of crystalline insulin brought about partial recovery, but death occurred four days later from cardiac infarction. —Authors' Summary.

A functioning tumor of the islands of Langerhans. Barnard, W. C., J. Path. & Bact. **35**: 929. 1932. Abst., Am. J. M. Sc. **186**: 149.

The author presents a case of an adenoma of the pancreas which appeared to have the function of secreting insulin. The patient complained of symptoms and presented signs of hypoglycemia, and was treated by considerable quantities of glucose. She recovered from several severe attacks but died 2 months later from the effect of hypoglycemia. Postmortem examination revealed an adenoma 1.2 cm. in diameter partly embedded in the head of the pancreas. The microscopic picture of this encapsulated mass led the author to believe this tumor to be an overgrowth of cells of the islets of Langerhans. It was considered benign, but locally invading its own capsule. The author is certain that if the patient had agreed to operation that removal of this functioning tumor would have resulted in a complete cure. It is pointed out, however, that the tumor would have been difficult to locate at operation, due to its position, but might have been found because of the firmer quality of the adenoma.

Effect of insulin on blood sugar of rabbits during infection. Fisher, L. C. and H. A. Reimann, Proc. Soc. Exper. Biol. & Med. **30**: 746. 1933.

Insulin administered during infection takes a little longer to produce its maximal effect, but the effect is more pronounced and lasts considerably longer. The effect on reduction of the blood sugar is in proportion to the gravity of the infection.—M. O. L.

The protection of insulin by antiproteases, and its absorption from the intestine. Harned, B. K. and T. J. Nash, Jr., J. Biol. Chem. **97**: 443. 1932.

By means of an antiprotease mixture prepared from Ascaris lumbricoides, the digestion of insulin by U. S. P. pancreatin, at pH 8, 37.5° C., could be inhibited completely for five hours, in vitro. Under otherwise identical conditions, but without antiprotease, insulin was inactivated immediately. Rabbits were used to test the mixtures for insulin activity before and after incubation. Insulin protected by a large excess of antiprotease was injected, through a small needle, directly into the duodenal lumen of both normal and depancreatized dogs. Approximately two inches of the duodenum was exteriorized permanently by sewing it into an incision through the abdominal wall. Animals were

allowed a minimum of seven days to recover from the operation. Normal dogs gave no response to insulin thus administered. Depancreatized dogs showed a decrease in urinary sugar and D:N ratios. Approximately 0.3% of the administered insulin was absorbed. All animals showed a normal response to subcutaneous injection. The small absorption of insulin from the duodenum under the conditions of the experiments is attributed to physical obstacles of dilution and diffusion imposed by the protein character of the hormone.—B. K. Harned.

The activation of insulin. Himsworth, H. P., Lancet, **223**: 935. 1932.

This article is a preliminary report of the work done by the author tending to show that insulin, before it is effective, must be activated by a substance which he has tentatively called insulin kinase. The problem was first approached through experiments on normal subjects. Results were obtained which proved that by altering the composition of the diet the efficiency of insulin in depressing the blood sugar could be varied at will. It was also demonstrated that there was a definite latent period after injection, during which the insulin exerted no measurable action. It was concluded that insulin as it is secreted by the pancreas in an inactive material which requires activating by some unknown substance (Insulin Kinase) in a manner somewhat analogous to the activation of a trypsinogen by entero-kinase. It is suggested that this unknown substance is produced by the liver. The details of the physiological results on normal subjects which have led to the formulation of this hypothesis are not given in this preliminary report.—H. C. Shepardson.

Uncontrollable causes of death in diabetic coma. Lande, H., J. A. M. A. **101**: 9. 1933. Abst., A. M. A.

The author states that vital statistics for New York City show that the steadily rising death rate for diabetes has not been checked by the introduction of insulin. This increasing mortality is a still unsolved problem in which the increased incidence of diabetes and the associated vascular disease probably play major parts. He has undertaken an analysis of the cases of diabetic coma occurring in adults during the insulin period for the purpose of investigating this phase of the problem. In the insulin period from 1923 to 1933, 82 cases of diabetic coma were treated in the adult wards of the Mount Sinai Hospital. There have been 24 deaths, a mortality of 29%. Two patients died in the early months of 1923 before an adequate supply of insulin was available. There were only 10 fatal cases of uncomplicated diabetic coma. Eight cases were associated with conditions fatal in themselves. In this group, coma was of only secondary importance and its control had no effect on the outcome. The age incidence is of the utmost importance. This group does not include children under 14 years of age, yet the mortality of the 49 patients under 40 years is 12% in contrast to that of 54% in the 33 patients over this age. The author believes that these figures are significant, particularly as the vital statistics of the city of New York show that the introduction of insulin has favorably affected the diabetic mortality only in the group under 35 years of age. Two factors, shock and hyperpyrexia, are of importance in the mechanism of fatal diabetic coma. The vasomotor collapse or shock presents the problem of restoration of blood volume. In a certain number of elderly persons, the circulatory collapse may persist and death may result even after carbohydrate metabolism is proceeding normally and the ketosis has been overcome. In this particular group, insulin does not prevent a fatal issue and there is unavoidable mortality despite adequate treatment with insulin and fluids. In those who died of hyperpyrexia, the etiologic factor was determined only three times. There is the possibility that one is dealing, in the remaining seven cases, with a terminal blood invasion, but in view of the negative blood culture in one case, this phenomenon, when it occurs, must be regarded as an essential part of diabetic coma, perhaps as a concomitant of dehydration. This type of hyperpyrexia may be of cerebral origin. In the author's experience, insulin has proved ineffective in the presence of hyperpyrexia. This is an added source of unavoidable mortality.

Hyperglycemia, glycosuria and the respiratory quotients as the result of insulinization of non-diabetics. Paul, W. D., B. B. Clark and Carol Martin, Proc. Am. Physiol. Soc. P. 79. 1933.

Non-diabetics receiving a general diet may be given insulin in gradually increasing amounts before meals, up to at least 75 units a day without hypo-

glycemia. Such patients have shown a hyperglycemia and glycosuria when the insulin courses were interrupted. Glucose tolerance curves are of the mildly diabetic type. Return to a normal state is progressive and requires several days. Determinations of the respiratory quotients show not only a lowered quotient after discontinuation of the insulin, but a progressively diminished R.Q. during the insulin course and a return to the normal figures paralleling or even more slowly than the recovery of the glucose tolerance.—Authors' Abst.

On the inheritance of diabetes mellitus; an analysis of 675 family histories. Pincus, G. and Priscilla White, Am. J. M. Sc. **186**: 1. 1933.

Histories were collected of 523 diabetic families and 153 non-diabetic. These were subjected to statistical analysis (with expert consultation). It appeared that incidences of diabetes were significantly higher in families of diabetic patients than in families of non-diabetic patients. If the assumption be accepted that potential diabetics, before they develop the disease, have the same expectations of life as persons generally, it was demonstrated that the observed occurrence of diabetes in the children of the diabetic families is in accord with the hypothesis that potentiality for developing diabetes is inherited as a simple Mendelian recessive. The probability that certain parents classed as non-diabetic are genetically diabetic would indicate that there is, on the hypothesis adopted, a deficiency of diabetic children in these diabetic families, but this presumed deficiency may be due to the limitations of the method of collecting family histories. In any event the data behave as if the identification of diabetics among the parents and children was in accord with Mendelian expectations, provided the calculations of the relative number of potential diabetics present in each decade are correct.—R. G. H.

The effect of previous hypophysectomy upon the diabetes resulting from pancreatectomy. Regan, J. F. and B. O. Barnes, Proc. Am. Physiol. Soc. P. 83. 1933.

The work of Houssay, who claimed that severe diabetes did not develop in dogs after pancreatectomy if the hypophysis had previously been removed was repeated. Two dogs lived only a short time after the second operation but in them the typical hyperglycemia which follows pancreatectomy did not develop. A third animal has now survived the second operation over 5 weeks and spontaneous glycosuria has not occurred. The blood sugar is normal or may be below normal on a short fast. Two tests with one-fourth unit of insulin per kilo resulted each time in convulsions which were relieved by glucose. The animal can tolerate 100 grams of glucose in 12 hours without any glycosuria. —Authors' Abst.

Effects of adrenal medulla and thyroid on the respiratory metabolism of pancreatic diabetes. Ring, G. C., and C. W. Hampel, Am. J. Physiol. **104**: 298. 1933.

Inactivation of the adrenal medulla failed to bring about a lowering of the high metabolic rate of diabetes. The average basal rate for four normal cats was 29.3 calories per square meter per hour. After removal of the pancreas the average rate increased to 41.7 calories. After inactivation of the adrenal medulla it was still 41.7 calories. Thyroidectomy in three cats did not prevent the usual increase in metabolism following pancreatectomy.—R. G. H.

The effect of parathyroid hormone on the calcium content of the milk of women (Die Wirkung des Parathyreoideahormons auf den Ca-Gehalt der Weiblichen Milch). Adamcsik, A. and A. von Beznak, Klin. Wchnschr. **10**: 2219. 1931.

There are daily variations in the calcium content of the milk of women, with a minimum in the morning and a maximum in the evening. Subcutaneous injections of parathyroid hormone (10-15 units of Squibb's preparation twice daily subcutaneously) led to a decrease of the daily variations. The calcium concentration of the milk tends to stay at the minimum level.—H. Selye.

The occurrence of guanidine as a contributory factor in parathyroid tetany in dogs. Bryan, W. R., A. S. Minot and L. L. Chastain, Proc. Am. Physiol. Soc. P. 13. 1933.

Previous investigations have shown that significant increases in guanidine concentration occur frequently but inconstantly in the blood of dogs during the

tetany which follows parathyroidectomy. In the present paper these inconstant results have been correlated with, and explained on the basis of, varying degrees of dehydration. Dehydration occurs frequently, especially in hot weather, following parathyroidectomy. This is due in part to the failure of experimental subjects to take, or retain, adequate amounts of water and also to increase fluid loss resulting from panting and salivation. Experiments have been carried out in which parathyroidectomized and normal control dogs were kept at different invironmental temperatures and were given varying amounts of fluid. The results show that when water is withheld from subjects kept at high environmental temperatures considerable increases in guanidine occur in the blood of both parathyroidectomized and control animals; when water is withheld at lower environmental temperatures this increase is less marked. In contrast to these findings when an adequate fluid intake is insured by the parenteral administration of fluid there is no increase in guanidine in the blood of either normal of parathyroidectomized dogs, even when they are exposed to high temperatures. These experiments indicate that parathyroid deficiency itself has no effect on the guanidine level of the blood; that dehydration is an important factor in determining whether or not hyperguanidinemia will occur following the removal of the parathyroid glands, and that increases in guanidine when they occur shorten the time of onset and increase the severity of tetany as was to be expected from the known antagonism between the action of guanidine bases and of calcium salts.—Authors' Abst.

Diagnosis and treatment of generalized osteitis fibrosa with hyperparathyroidism. Elmslie, R. C., F. R. Fraser, T. P. Dunhill, R. M. Vick, C. F. Harris and J. A. Dauphinee, Brit. J. Surg. **20**: 479. 1933. Abst., J. A. M. A. **100**: 1727.

The authors report three cases of generalized osteitis fibrosa with hyperparathyroidism, with an account of the bony lesions as determined by exploratory operations and roentgen examinations, and the results of a chemical investigation of the calcium and phosphorus metabolism. In each of three cases a tumor of the parathyroids was removed by operation. Tetany resulted from the operation in two of the cases. The diagnosis of generalized osteitis fibrosa depends on the demonstration of generalized rarefaction of the bones, in addition to the presence of cystlike areas of rarefaction, cellular tumors of the bones, spontaneous fractures, muscular weakness and wasting, and aches and pains in the limbs. Renal calculi were present in two of the three cases on roentgen examination. An abnormally high level of serum calcium was not always found, even when a parathyroid tumor was present. In the authors' three cases an increased excretion of calcium in the urine was constantly demonstrated. In cases of generalized osteitis fibrosa, the presence of hyperparathyroidism should be demonstrated before operation on the parathyroids is undertaken. After the removal of the parathyroid tumor, the level of serum calcium fell strikingly and calcium was retained by the body; or, if retention was present previously, this was definitely increased. An abnormal condition of the phosphorus metabolism was found in each of the three cases, but no consistent relation could be demonstrated between the changes in the calcium metabolism and the phosphorus metabolism, either before or after the removal of the parathyroid tumors. As a result of the removal of the parathyroid tumor, striking improvement in general health and strength and cessation of pain resulted in each of the three cases, and no further fractures occurred. A considerable increase in the density of the bones, especially around the cysts and in the cortical bones, was seen from 10 to 24 months after the operation. In one case the appearance of the skull showed a striking return toward the normal 20 months after the operation.

Parathyroid hormone—osteitis fibrosa—kidney stone (Parathormon-Osteitis fibrosa—Nierenstein). Mandel, F. and R. Uebelhör, Zentralbl. f. Chir. **60**: 68. 1932.

Investigations of the last few years have shown that osteitis fibrosa is due to hyperfunction of the parathyroids. The object of this paper is to show that the kidney stones which have been frequently observed as a complication of this disease are also due to hyperparathyroidism. The authors injected 10 units of parathyroid hormone into guinea-pigs over a period of four weeks and found calcium cylinders in the kidneys at autopsy. They conclude that stone formation in osteitis fibrosa is the result of disturbance in calcium metabolism due to a hyperfunction of the parathyroids.—H. Selye.